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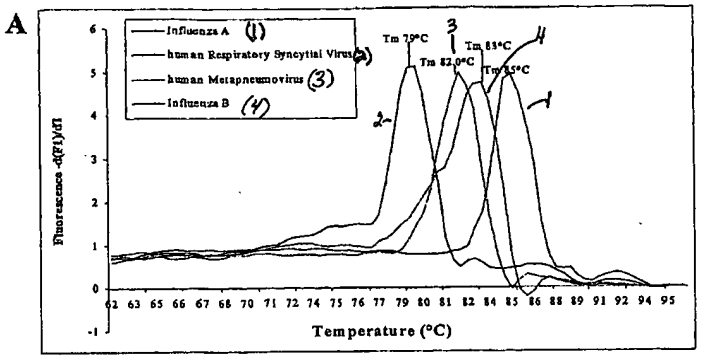

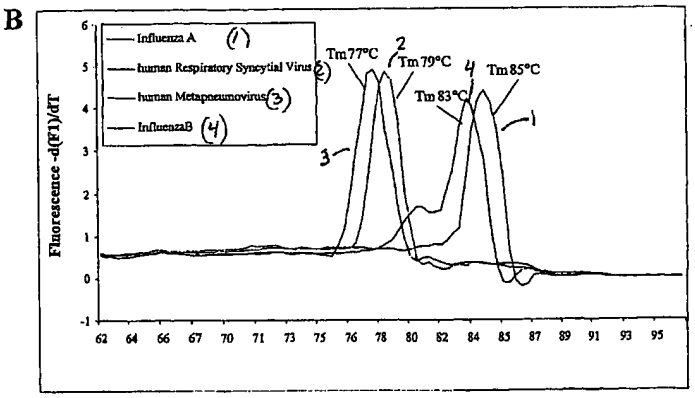

(57) Abstract: There is provided compositions and methods for the detection and/or quantification of respiratory viruses using specific nucleic acid sequences. In particular methods and composition are provided to detect human Metapneumovirus (hMPV). The invention also provides with multiplex assays for detecting respiratory viruses in test samples.

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MOLECULAR METHODS AND COMPOSITIONS FOR DETECTING AND QUANTIFYING RESPIRATORY VIRUSES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority on Canadian applications serial number 2,411,264 filed on December 19, 2002 entitled "Sequences for detection and identification of the human metapneumovirus" and serial number 2,418,004 filed on January 24, 2003 entitled "Multiplex assays for detection and identification of viral respiratory pathogens.

TECHNICAL FIELD

This application relates to the field of microrganisms detection and more particularly to the detection of respiratory tract viruses using virus specific nucleic acids.

BACKGROUND OF THE INVENTION

Respiratory tract infections are a significant cause of morbidity and mortality in all age groups but especially in young children, elderly subjects, and immunocompromised patients. Most of these infections are caused by viruses with influenza A and B and the human respiratory syncytial virus (hRSV) being associated with the most frequent and severe complications i.e. pneumonitis and death (Dowell et. al., 1996, J. Infect. Dis. 174:456-462; Simonsen et al., 2000, J. Infect. Dis. 181:831-837; Han et al., 1999, J. Infect. Dis. 179:25-30; Neuzil et al., 2000, N. Engl. J. Med. 342:225-231; Simoes, 1999, Lancet 354:847-852.). Other viruses such as adenoviruses (> 50 serotypes), parainfluenza viruses (PIV) (4 serotypes), rhinoviruses (> 100 serotypes), and coronaviruses also contribute to acute respiratory tract infections (Treanor, 2002, Clinical Virology, 2nd ed., ASM Press, Washington, D.C., 7-26). Also, recent studies by the inventor and others have suggested that the new human metapneumovirus (hMPV) should be added to the list of human respiratory viral pathogens in all age groups (van den Hoogen et al., 2001, Nat. Med. 7:719-724;

Peret et al., 2002, J. Infect. Dis. 185:1660-1663; Boivin et al., 2002, J. Infect. Dis.186:1330-1334).

The hMPV was initially isolated from nasopharyngeal aspirates recovered from 28 children suffering from respiratory tract infections. Based on partial nucleic acid sequences, gene organization, and electron microscopy (EM) findings, this virus was preliminary assigned to the *Paramyxoviridae* family, subfamily *Pneumovirinae*, genus *Metapneumovirus*. Since then, hMPV has been isolated in North America by our group (Peret et al., 2002, J. Infect. Dis. 185:1660-1663), in Australia (Nissen et al., 2002, Med. J. Aust. 176:188), and in England (Stockton et al., 2002, Emerg. Infect. Dis. 8:897-901).

Preliminary virological characteristics of hMPV.

The initial report by Van Den Hoogen *et al.* (van den Hoogen et al., 2001, Nat. Med. 7:719-724) indicated that the new virus replicated slowly in tertiary monkey kidney (TMK) cells with cytopathic effects (CPE) consisting of syncytium formation appearing 10-14 days post inoculation. Notably, the virus grew very poorly in Vero and A549 cells and could not be propagated in Madin Darby canine kidney (MDCK) cells. Virus-infected TMK cell-culture supernatants also did not display any hemagglutinating activity. The supernatants of infected TMK cells were used for EM studies, which revealed the presence of paramyxovirus-like pleomorphic particles in the range of 150-600 nm with short envelope projections in the range of 13-17 nm. However, RT-PCR studies with primer sets specific for known paramyxoviruses were initially all negative. Using a random PCR amplification procedure known as RAP-PCR (Welsh et al., 1990, Nucleic Acids Res. 18:7213-7218; Ralph et al., 1993, Proc. Natl. Acad. Sci. U.S.A. 90:10710-10714), the Dutch investigators identified differentially displayed bands (compared to human parainfluenza-1 virus) which, upon sequencing, were found to be most closely related to the avian pneumovirus (APV).

APV, the agent of turkey rhinotracheitis, has been the sole member of the recently assigned *Metapneumovirus* genus within the *Pneumovirinae* subfamily (Taxonomy Virus, 2000, Seventh Report of the International Committee on Taxonomy of Viruses. San Diego. 551, 557, 559-560). The latter virus is a major respiratory pathogen of turkeys and other domestic fowl worldwide (Yu et al., 1992, Virology. 186:426-434; Jones et al., 1987, Vet. Rec. 120:301-302; Jones, 1996, Avian Pathol. 25:639-648; Seal et al., 2000, Virus Res. 66:139-147). There are four serotypes of APV with serotype C (present in US) being the most closely related to hMPV (van den Hoogen et al., 2001, Nat. Med. 7:719-724). The metapneumoviruses (including APV and hMPV) are structurally similar to the human respiratory syncytial virus (hRSV), which also belongs to the *Pneumovirinae* subfamily, except for two features: they lack the two non-structural proteins (NS1 and NS2) and they have a different genomic organization (Yu et al., 1992, Virology. 186:426-434). Recently, an almost complete hMPV sequence (13.4 kb) was reported in GenBank (accession number: AF371337) (van den Hoogen et al., 2002, Virology. 295:119-132). The overall percentage of amino acid sequence identity for the N (nucleoprotein), P (phosphoprotein), M (matrix), F (fusion), M2 (membrane), and L (polymerase) proteins is in the range of 56-88% between hMPV and APV whereas it is 18-44% between hMPV and hRSV. However, the G (glycoprotein) and SH (small hydrophobic) proteins of hMPV do not possess significant amino acid sequence identity with those of any other viruses. In contrast to APV, hMPV was shown to be unable to replicate in turkeys and chickens but the human virus replicated efficiently in the respiratory tract of cynomolgous macaques inducing upper respiratory tract symptoms that correlated with peak viral replication in throat samples between 2 to 8 days post infection (van den Hoogen et al., 2001, Nat. Med. 7:719-724). Thus, these preliminary data indicated that hMPV was a primate pathogen associated with respiratory diseases.

Clinical importance of hMPV.

There is little information about the clinical presentation of hMPV infection. In the original report by Van Den Hoogen *et al.*, 27 of the 28 infected children from the Netherlands were below the age of five (van den Hoogen et al., 2001, Nat. Med. 7:719-724). It was mentioned that those infected patients were diagnosed with a range of acute respiratory tract infections (ARTI) from mild to severe including bronchiolitis and pneumonia although no detailed clinical data were provided. In a limited study conducted in the winter of 2000, the same authors found that 7 (≈ 10%) of 68 pediatric patients with unexplained ARTI had evidence of acute hMPV infection by RT-PCR (van den Hoogen et al., 2001, Nat. Med. 7:719-724). In addition, Australian investigators reported the detection of hMPV by RT-PCR in 3/200 (1.5%) nasopharyngeal aspirates from children whose specimens were negative for the common respiratory viruses during the winter of 2001 (Nissen et al., 2002, Med. J. Aust. 176:188). Recently, hMPV RNA was detected by RT-PCR in 9/405 (2.2%) of nasopharyngeal swabs from English patients who consulted for influenza-like illnesses and had negative tests for other pathogens (Stockton et al., 2002, Emerg. Infect. Dis. 8:897-901). These studies, however, suffer from major limitations such as their small size (in most cases), the superficial description of clinical features, the lack of morbidity/mortality data, their retrospective nature, the exclusion of patients who tested positive for other viruses, and the absence of a control group.

Thus far, seroprevalence studies have been limited to the Dutch population. An indirect immunofluorescence assay (IFA) using infected TMK cells has revealed that 25% of the children aged between 6 and 12 months had hMPV antibodies and 70% had become seropositive by the age of 5 years (n=60) (van den Hoogen et al., 2001, Nat. Med. 7:719-724). Retrospective serological testing also indicates that hMPV has been circulating in the Dutch population for at least half a century.

Diagnosis of hMPV infections

Our laboratory was the first to report the presence of hMPV in America and the second in the world following the initial report from the Netherlands (Peret et al., 2002, J. Infect. Dis. 185:1660-1663). Our first 11 hMPV strains were isolated on LLC-MK2 cells, a continuous cell line from rhesus monkey kidneys that is used in our center to recover parainfluenza viruses (PIV). Electron microscopy studies of these isolates revealed pleomorphic, spherical and filamentous particles with characteristics (nucleocapsid diameter and pitch spacing, length of projections) that were consistent with paramyxoviruses. RT-PCR and immunofluorescent assays for paramyxoviruses (PIV 1-3, hRSV) and other respiratory viruses (adenoviruses, coronaviruses, influenza viruses A and B, rhinoviruses) were negative for all 11 isolates. In contrast, amplification of the hMPV F (fusion) and M (matrix) genes based on published partial sequences (van den Hoogen et al., 2001, Nat. Med. 7:719-724; van den Hoogen et al., 2002, Virology. 295:119-132) was positive for all isolates (Peret et al., 2002, J. Infect. Dis. 185:1660-1663).

Subsequently, the virological features and clinical findings associated with a larger set of 38 hMPV strains from subjects hospitalized at our institution (CHUL, Québec City) since 1993 were examined in our laboratory (Boivin et al., 2002, J. Infect. Dis. 186:1330-1334). With all cell lines used in our laboratory, we were able to determine that the previously unidentified hMPV strains only consistently grew on LLC-MK2 cells after a mean incubation time of 17.3 days (range 3-23 days). Interestingly, most isolates recovered before 2002 produced only limited syncytia on LLC-MK2 cells, whereas some of the recent strains produced large syncytia typical of those induced by hRSV. Notably, we also observed a seroconversion for hMPV in two adult patients for whom acute and convalescent sera were available using an IFA test designed in our laboratory. Such seroconversion episodes (titers from < 1/10 to 1/80 and from < 1/10 to 1/160 over 2 and 4 weeks, respectively) occurred during the course of the patients' illness suggesting that hMPV was a true respiratory pathogen. A phylogenetic analysis of hMPV Canadian strains based on F gene sequences identified two major phylogenetic groups. At the nucleotide level, we found a similarity of 94.2-100% and 92.6-100% for isolates of groups 1 and 2, respectively, whereas the similarity at the amino acid level was higher i.e. 96.8-100% (group 1) and

97.2-100% (group 2) suggesting a functional/immunological conservation of the F protein. Interestingly, co-circulation of the two hMPV groups during some years was observed.

Over the past 9 years, most (86.7%) hMPV strains from our center were isolated during a 5-month period, from December to May. As an indication of the relative frequency of detection of hMPV compared to other respiratory viruses, we reviewed all respiratory samples (n = 862) sent to our virology laboratory for viral culture during the winter season (Dec. to May) of 2000-01. During this period, hMPV was recovered in 2.3% of all respiratory samples tested by viral culture compared to 2.3% for PIV, 3.4% for adenoviruses, 4.8% for hRSV, and 19.8% for influenza A or B viruses. Overall, hMPV represented 7.1% of all positive cultures from respiratory samples during that winter season with percentages of 4.1%, 2.8%, and 29.3% for patients aged 0-5, 5-65, and > 65 years, respectively. A retrospective review of the clinical charts from hMPV-infected subjects identified for the first time the role of this virus as a cause of severe lower respiratory tract infections in all age groups but especially in young children, elderly subjects, and immunocompromised patients. More specifically, the most frequent diagnoses associated with hMPV infection in hospitalized children were pneumonitis (66%) and bronchiolitis (58.3%) whereas bronchitis/bronchospasm (60%) and pneumonitis (40%) were most commonly seen in elderly subjects (Boivin et al., 2002, J. Infect. Dis. 186:1330-1334). Although no histopathological studies were available, hMPV was the only pathogen identified in respiratory tract secretions of 3 subjects (including 2 with immunosuppressive conditions) who died of respiratory insufficiency.

We recently studied in greater details one of the cases associated with a fatal hMPV infection (Pelletier et al., 2002, Emerg. Infect. Dis. 8:976-978). Interestingly, this child with acute lymphoblastic leukemia was successively infected by two genetically-distinct hMPV strains on two consecutive winter seasons. On each occasion, hMPV was the only pathogen isolated from a nasopharyngeal aspirate obtained during the course of lower respiratory tract infections (bronchiolitis in the

first year, bronchiolitis followed by fatal pneumonitis in the second year). Comparative sequence analysis of the amplified F gene products (759 bp) revealed that the two viral isolates differed by 114 nucleotides (10 amino acids) and belonged to two different hMPV groups. Overall, our data suggest that many hMPV strains may co-circulate in a specific population during a given period and such viral diversity coupled with a waning immunity (as found in elderly subjects and immunocompromised patients) may lead to multiple and sometimes fatal reinfections similar to those reported with hRSV (Hall, 2001, N. Engl. J. Med. 344:1917-1928; Couch et al., 1997, Am. J. Med. 102:2-9).

The role and relative contribution of hMPV were recently further expanded in a prospective case-control study performed at our institution during the winter of 2001-02 (Dec. 15-April 20). All children ≤ 3 years were eligible as cases if they were hospitalized for a lower respiratory tract infection (bronchiolitis, croup, pneumonitis) whereas controls consisted of children matched for age and hospitalized for an elective surgery during the same period with no respiratory symptoms. Nasopharyngeal aspirates were obtained from all participants for real-time PCR studies performed on a LightCycler (Roche). The latter included a multiplex RT-PCR for common respiratory viruses (influenza A, influenza B, hRSV) and a real-time RT-PCR assay for hMPV (analytical sensitivity of 100 copies per PCR reaction). No viruses were detected in samples from the 51 control patients. In contrast, at least one virus was identified in samples from 163 (78.4%) of the 208 cases (51% hRSV, 21.6% influenza A, 0% influenza B, 5.8% hMPV, and 5.9% co-infections). hRSV and influenza A infections occurred predominantly in the period between January and March 2002, whereas hMPV infections increased after February. In fact, 11/12 hMPV cases occurred within a 4-week period from mid-March to mid-April accounting for 18.9% of all ARTI in children during this period. Data collected from our virology laboratory indicated that the hMPV outbreak extended after the end of the study protocol (April 20) which suggests that hMPV seasonal pattern may differ from that of other respiratory viruses. A bronchiolitis was diagnosed in 67% of hMPV-infected children whereas 16.7% developed a pneumonia. In brief, there was no statistical

difference between the different viruses with regard to the symptomatology and reported diagnoses. Similarly, the median duration of hospitalization for the 3 viruses was identical (4-5 days).

In view of the above, it will be appreciated that diagnosis assay that can rapidly detect the presence of many respiratory viruses is needed.

Respiratory viruses have been classically identified by viral culture using a variety of permissive cell lines. However, viral culture is not convenient for clinical management due to the need of specialized techniques (cell culture, immunofluorescence methods), the long turnaround time before appearance of typical cytopathic effects for many viruses (up to a few weeks), and the need for rapid inoculation of an infectious virus into multiple cell lines for optimal sensitivity (Specter et al., 2002, Clinical Virology, 2nd ed., ASM Press, Washington, D.C., 243-272).

Antigenic detection kits for some respiratory viruses (influenza viruses and hRSV) have been developed by many companies. Most are immunoenzymatic assays (ELISA) based on the capture of a viral antigen by a specific monoclonal antibody. Although such assays eliminate many of the problems inherent to viral cultures (no need for specialized equipment, rapid turnaround time in < 30 min, no need to recover an infectious virus), they have not been widely adopted due to poor sensitivity and/or specificity compared to viral culture. For instance, the sensitivity and specificity of some rapid antigenic tests for influenza has varied from 46 to 96 % and from 52 to 99 %, respectively, compared to viral culture. The performance of these tests is dependent on many variables such as the patient's age, the type of specimen and the duration of the disease (Hayden et al., 2002, Clinical Virology, 2nd ed., ASM Press, Washington, D.C., 891-920). The performance of the rapid antigenic tests for hRSV has been considered quite good in children with sensitivity and specificity varying from 80 to 95 % (Piedra et al., 2002, Clinical Virology, 2nd ed., ASM Press, Washington, D.C., 763-790) but poor in adults (Englund et al., 1996, J. Clin.

Microbiol. 34:1649-1653). Detection of antibodies against viral pathogens (serology) has a limited value in the diagnosis of acute viral infections since two serums, collected at a 2 to 3 week interval, are required in order to document a significant rise in viral antibody titers.

More recently, PCR or RT-PCR assays have been developed for many respiratory viruses allowing detection of small amounts of viral DNA or RNA in a clinical sample. In the so-called "multiplex" format, PCR assays have been designed to amplify more than one viral target in the same PCR reaction (Ellis et al., 1997, J. Clin. Microbiol. 35:2076-2082; Fan et al., 1998, Clin. Infect. Dis. 26:1397-1402; Liolios et al., 2001, J. Clin. Microbiol. 39:2779-2783; Grondahl et al., 1999, J. Clin. Microbiol. 37:1-7). However, most multiplex PCR assays reported to date do not allow the simultaneous detection of all viruses involved in acute respiratory tract infections of humans and they require separate steps for the amplification and detection of viral genes, greatly increasing the assay's turnaround time and preventing reliable quantification of the amplicons. At present, only one multiplex PCR assay (the Hexaplex test from Prodesse, Waukesha, WI) is commercially-available. The Hexaplex detects 6 respiratory viruses (influenza A and B, PIV 1-3 and hRSV) using specific viral probes in a microplate format (Fan et al., 1998, Clin. Infect. Dis. 26:1397-1402; Kehl et al., 2001, J. Clin. Microbiol. 39:1696-1701). This assay does not use real-time amplification methodologies and has a turnaround time of about one full working day. Thus, there is a need for developing more rapid molecular assays that permit detection of a larger panel of respiratory viral pathogens.

Rapid and sensitive detection of respiratory viruses is an important clinical goal to help physicians select the most appropriate antiviral, reduce nosocomial transmission of pathogens, decrease the length of hospitalization, and reduce antibiotic use and bacterial resistance. The present invention relates to the development of multiplex assays based on nucleic acids detection for sensitive and rapid diagnosis of most viral respiratory pathogens of humans.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a rapid and sensitive method using amplification primers and/or probes for determining the presence and/or the amount in a test sample of nucleic acids from clinically important respiratory viral pathogens including human metapneumovirus.

In another aspect there is provided multiplex assays aimed at detecting conserved genes of common respiratory viral pathogens such as influenza A and B, human respiratory syncytial virus (hRSV) A and B, human metapneumovirus (hMPV), parainfluenza viruses (PIV) types 1-4, adenoviruses, rhinoviruses, enteroviruses, and coronaviruses.

The nucleic acid amplification method is preferably but not exclusively PCR. In a preferred embodiment, identification of the amplicons produced by the amplification of nucleic acids from respiratory viruses is achieved by verifying the specific melting temperature of amplified viral products or by hybridization with specific viral probes. Other amplification technologies including target and probe amplification techniques as well as signal amplification techniques performed in liquid phase or onto solid supports may also be used.

It is an object of the present invention to provide a method using nucleic acid amplification and/or detection for determining the presence and/or the amount of nucleic acids of the human metapneumovirus (hMPV).

In a preferred embodiment, the nucleic acid amplification method is PCR. However, other amplification technologies including target and probe amplification techniques as well as signal amplification performed in liquid phase or onto solid support may also be used.

This invention provides a method to detect the presence of hMPV in a test sample based on the detection of a nucleotide sequence from either the nucleocapsid (N), phosphoprotein (P), matrix (M), fusion (F), membrane (M2), polymerase (L), glycoprotein (G), and small hydrophobic (SH) genes. In a particularly preferred embodiment, the method to detect the presence of hMPV in a test sample is based on the detection of a nucleotide sequence from the nucleoprotein (N) or polymerase (L) genes.

In another embodiment, identification of amplified hMPV products is achieved by verifying the specific melting temperature of amplified viral products or by hybridization with hMPV-specific probes.

It is another object of this invention to provide a method for typing hMPV strains which comprises the steps of reproducing the above amplification method followed by either hybridization using type-specific probes, DNA sequencing or restriction enzyme analysis. Typing is done preferentially, but not exclusively, on the most variable hMPV genes i.e. the fusion (F) and glycoprotein (G) genes.

All the proprietary DNA fragments and oligonucleolides useful in the detection or identification of a hMPV strain are further objects of this invention as well as any composition of matters that include them (recombinant vectors, hosts, diagnostic kits...).

BRIEF DESCRIPTION OF THE DRAWINGS

Further features and advantages of the present invention will become apparent from the following detailed description, taken in combination with the appended drawings, in which:

Figure 1 represents a melting curve analysis of amplified hMPV strains using specific nucleoprotein primers (Sequence ID Nos. 92 and 94) ;

Figure 2 shows an ethidium bromide-stained agarose gel showing restriction fragment length polymorphism of amplified hMPV fusion (F) gene sequences digested with enzymes ApaL I and Avr II.PCR products of the hMPV F gene (amplified with primers sequence ID Nos. 97 and 98) were digested with ApaL I and Avr II. Strains belonging to hMPV genotype F-1 were digested only by ApaL I resulting in two fragments of 532 bp and 227 bp whereas strains belonging to hMPV genotype F-2 were digested only by Avr II resulting in fragments of 447 and 312 bp.;

Figure 3 shows the age at admission of children hospitalized for acute respiratory tract infections (ARTI) caused by hMPV (A), hRSV (B), and influenza A (C) as well as for the whole study population (D) described in example 4;

Figure 4 represents the biweekly distribution of virologically cases with acute respiratory tract infections and their controls in the study reported in example 4;

Figure 5 represents the biweekly distribution of hMPV isolates in the study group (hospitalized children) described in example 4 and in respiratory specimens tested at the regional virology laboratory (RVL);

Figure 6 illustrates a phylogenetic tree of hMPV strains recovered as part of the study described in example 4 also including the prototype strain from the Netherlands (GenBank af371367) and a Canadian isolate from season 2000-01 (hMPV35-2001). The dates of sample collection are in parentheses;

Figure 7 is a melting curve analysis of viral strains amplified using the respiratory-1 multiplex real-time PCR assay. A- Multiplex real-time PCR assay using hMPV N primers (SEQ ID NOS. 95 and 96). B- Multiplex real-time PCR assay using hMPV L

primers (SEQ ID NOS. 112 and 114). Note: the internal control is apparent (Tm: 91°C) in the absence of amplification of specific viral products.;

Figure 8 is a melting curve analysis of viral strains amplified using the respiratory-2 multiplex real-time PCR assay. Note: The internal control is apparent (Tm: 91°C) in the absence of amplification of specific viral products;

Figure 9A is a fluorescence signal as a function of elongation step for the determination of the hMPV load in clinical samples using quantitative real-time PCR;

Figure 9B is a standard curve for determination of the hMPV using quantitative real-time PCR;

Figure 10A is a phylogenetic tree for the F gene of hMPV;

Figure 10B is a phylogenetic tree for the G gene of hMPV;

Figure 11 is a melting curve analysis of amplified viral genes and of an internal control as determined by the LightCycler instrument. Note that high amounts of amplified viral products may preclude detection of the internal control in some PCR runs; and

Figure 12 is a melting curve analysis SARS-coronavirus using a real time PCR assay.

It will be noted that throughout the appended drawings, like features are identified by like reference numerals.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

The invention provides nucleic acid based diagnostic methods and compositions to detect and quantify respiratory viruses.

In a preferred embodiment respiratory viral pathogens targeted by the diagnostic assays include all genotypes of influenza A and B viruses, hRSV, hMPV, PIV, adenoviruses, rhinoviruses, and enteroviruses. Other less common (coronaviruses, reoviruses) and yet to be discovered respiratory viruses are also targets under the scope of the present invention.

In the description of this invention, the terms «nucleic acids» and «sequences» might be used interchangeably. However, «nucleic acids» are chemical entities while «sequences» are the pieces of information encoded by these «nucleic acids». Both nucleic acids and sequences are equivalently valuable sources of information for the matter pertaining to this invention.

In one embodiment the method targets conserved hMPV nucleotide sequences for diagnostic purposes and variable hMPV sequences for epidemiological typing purposes. Nucleotide sequences were selected either from a number of hMPV viral sequences obtained in the inventor's laboratory or from public databases (GenBank accession number AF371337).

Our proprietary DNA fragments and oligonucleotides (a non-restrictive list of primers and probes) are listed in the following tables.

It will be understood by those skilled in the art that sequences that can selectively hybridize to the sequences of the present invention are also comprised in the scope of the invention. By selectively hybridizing it is meant that a nucleic acid molecule binds to a given target in a manner that is detectable in a different manner from non-target

sequence under moderate, or more preferably under high, stringency conditions of *hybridization*. "Complementary" or "target" nucleic acid sequences refer to those nucleic acid sequences which selectively hybridize to a nucleic acid molecule. Proper annealing conditions depend, for example, upon a nucleic acid molecule's length, base composition, and the number of mismatches and their position on the molecule, and must often be determined empirically. For discussions of nucleic acid molecule (probe) design and annealing conditions, see, for example, Sambrook et al. 1989 (Sambrook, et al., Molecular Cloning: A Laboratory Manual, 2d Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)) or Ausubel et al. 1987 (Ausubel, F., et al., Current Protocols in Molecular Biology, New York (1987)).

The detection of the viruses including the hMPV is conducted through compositions of matters such as diagnostic kits comprising the amplification primers or probes of this invention.

In the methods and kits of the present invention, probes and primers are not limited to nucleic acids and may include, but are not restricted to, analogs of nucleotides. The diagnostic reagents constituted by the probes and the primers may be present in any suitable form (bound to a solid support, liquid, lyophilized, etc.) for detection and/or amplification of nucleic acid sequences of the viruses.

In the methods and kits of the present invention, amplification reactions may include but are not restricted to: a) polymerase chain reaction (PCR), b) ligase chain reaction (LCR), c) nucleic acid sequence-based amplification (NASBA), d) self-sustained sequence replication (3SR), e) strand displacement amplification (SDA), f) branched DNA signal amplification (bDNA), g) transcription-mediated amplification (TMA), h) cycling probe technology (CPT), i) nested PCR, j) multiplex PCR, k) solid phase amplification (SPA), l) nuclease dependent signal amplification (NDSA), m) rolling circle amplification technology (RCA), n) anchored strand displacement amplification, o) solid-phase (immobilized) rolling circle amplification.

In the methods and kits of the present invention, detection of the nucleic acids of target genes may include real-time or post-amplification technologies. These detection technologies can include, but are not limited to, the use of intercalating agents such as SYBR green, fluorescence resonance energy transfer (FRET)-based methods such as adjacent hybridization of probes (including probe-probe and probe-primer methods), *Taq*Man probe, molecular beacon probe, Scorpion probe, nanoparticle probe and Amplifluor probe. Other detection methods include target gene nucleic acids detection via immunological methods, solid phase hybridization methods on filters, chips or any other solid support. In these systems, the hybridization can be monitored by fluorescence, chemiluminescence, potentiometry, mass spectrometry, plasmon resonance, polarimetry, colorimetry, flow cytometry or scanometry. Nucleotide sequencing, including sequencing by dideoxy termination or sequencing by hybridization (e.g. sequencing using a DNA chip) represents another method to detect and characterize the nucleic acids of target genes.

Amplification of hMPV sequences

In one aspect of the invention there is provided methods and compositions for detecting hMPV in a sample.

In a preferred embodiment, a reverse-transcription (RT)-PCR protocol is used for nucleic acid amplification. The single-stranded viral RNA is converted to complementary DNA using an enzyme possessing a reverse transcriptase activity and a specific hMPV primer. Alternatively, random hexamer primers can be substituted to the specific hMPV primer. In a preferred embodiment, viral RNA was prealably extracted from 200 µl of naso-pharyngeal aspirates using the QIAamp Viral RNA Mini Kit (QIAGEN). Complementary DNA was synthesized using 10 µl of eluted RNA, 0.75 µM of a specific hMPV primer (Table 2, sequences ID Nos. 91, 97, 100, 102, 109, 112), and the Omniscript Reverse Transcriptase Kit (QIAGEN, Mississauga, ON, Canada) following the manufacturer's recommendations.

For DNA amplification by the widely used PCR method, primer pairs were derived from our proprietary DNA fragments or from public database sequences (Table 1). During DNA amplification by PCR, two oligonucleotide primers binding respectively to each strand of the heat-denatured target DNA from the microbial genome are used to amplify exponentially *in vitro* the target DNA by successive thermal cycles allowing denaturation of the DNA, annealing of the primers and synthesis of new targets at each cycle (Persing, 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, DC. 88-109).

In a preferred embodiment, complementary DNA was amplified using a real-time PCR procedure with the LC Faststart DNA Master SYBR Green 1 Kit (Roche Diagnostics, Laval, QC, Canada) or the SYBR Green TAQ ReadyMix for quantitative PCR capillary formulation (Sigma-Aldrich, Oakville, ON) in a LightCycler instrument (Roche Diagnostics). Each reaction had a total volume of 20 µl including 2 µl of RT mixture and 18 µl of a reaction mixture containing 4 mM $MgCl_2$, 2 µl of Faststart DNA SYBR Green Master Mix or the SYBR Green TAQ ReadyMix for quantitative PCR capillary formulation, 3% DMSO, and 0.5 µM of each hMPV primer (see Tables 2 and 4 for sequence ID Nos. and specific combinations of primers for amplification, respectively). Cycling conditions typically included an initial denaturation step of 10 min. at 94°C, followed by 50 cycles of 15 s at 94°C, 5 s at 54°C, and 30 s at 72°C. However, the cycling conditions could slightly vary according to the hMPV gene to amplify.

The person skilled in the art of nucleic acid and/or signal amplification knows the existence of other rapid amplification procedures such as ligase chain reaction (LCR), reverse transcriptase PCR (RT-PCR), transcription-mediated amplification (TMA), self-sustained sequence replication (3SR), nucleic acid sequence-based amplification (NASBA), strand displacement amplification (SDA), branched DNA (bDNA), cycling probe technology (CPT), solid phase amplification (SPA), rolling circle amplification technology (RCA), solid phase RCA, anchored SDA and nuclease dependent signal amplification (NDSA) (Persing, 1993, Diagnostic Molecular

Microbiology: Principles and Applications, American Society for Microbiology, Washington, DC. 88-109; Lee, 1997, Nucleic Acid Amplification Technologies: Applications to Disease Diagnosis, Eaton Publishing, Boston, MA. 61-125; Westin et al., 2000, Nat. Biotechnol. 18:199-204). The scope of this invention is not limited to the use of amplification by PCR, but rather includes the use of any nucleic acid amplification method or any other procedure which may be used to increase the sensitivity and/or the rapidity of nucleic acid-based diagnostic tests. The scope of the present invention also covers the use of any nucleic acid and/or signal amplification and detection technology including real-time or post-amplification detection technologies, any amplification technology combined with detection, any hybridization nucleic acid chips or array technologies, any amplification chips or combination of amplification and hybridization chip technologies. Detection and identification by any nucleotide sequencing method is also under the scope of the present invention.

Any oligonucleotide, useful for diagnosis, and which are derived from hMPV sequences and used with any nucleic acid amplification and/or hybridization technologies are also under the scope of this invention.

Detection of amplification products

Classically, the detection of PCR amplification products is performed by standard ethidium bromide-stained agarose gel electrophoresis. Using this approach, primers specific for a nucleotide sequence from either the nucleocapsid (N), phosphoprotein (P), matrix (M), fusion (F), polymerase (L), glycoprotein (G), genes generated amplicons of various lengths depending on the selected primers pairs (Table 4). It is however clear that other detection methods of amplified products may be faster, more sensitive, and more practical. Amplicon detection may also be performed by solid support or liquid hybridization using strain-specific internal DNA probes hybridizing to an amplification product. Such probes may be generated from sequences from our repertory (Table 1) and designed to specifically hybridize to DNA amplification

products which are objects of the present invention. Amplicons can also be characterized by DNA sequencing. Alternatively, a rapid detection method developed with real-time PCR assays consists of determining the specific melting temperature of amplicons at the end of the amplification reaction.

In a preferred embodiment, detection of hMPV amplicons was characterized using the melting curve analysis feature of a real-time PCR instrument. Briefly, following the last amplification cycle on a LightCycler instrument, the internal temperature was rapidly increased to 94°C then decreased to 60°C for 30 seconds, followed by slowly increasing to 94°C at a rate of 0.1°C per second, with continuous fluorescence lecture. Using this procedure, the specific melting temperature obtained with the specific amplicons produced by the pairs of primers specific for the N (SEQ ID No 92+94), M (SEQ ID No 100+101), F (SEQ ID 97+98), P (SEQ ID No 102 +103) and L (SEQ ID No 112 + 113) gene sequences was found to be 82.63°C ± 0.87°C, 82.75°C ± 0.80°C, 83.29°C ± 0.45°C, 80.48°C ± 0.46°C, and 77.99 ± 0.33°C, respectively (Table 5 and see also Figure 1 for example of the melting temperature obtained for different hMPV strains with the N primers). Alternatively, we also designed specific adjacent and TaqMan fluorescent probes for the various hMPV gene products (N, M, F, G, P and L) that can be used for real-time detection and quantification of amplicons (Table 3). Here, quantification of hMPV gene products is achieved through fluorescence resonance energy transfer (FRET) when the two probes hybridize nearby on a complementary DNA sequence. Other types of detection methods including the use of TaqMan or molecular beacon probes specific for the hMPV sequences described above are also under the scope of this invention.

Typing of hMPV

Previous studies have shown that two major genotypes of hMPV circulate in North America and Europe (van den Hoogen et al., 2001, Nat. Med. 7:719-724; Boivin et al., 2002, J. Infect. Dis. 186:1330-1334). We also designed a rapid molecular typing method for epidemiologic studies based on amplification of a portion of the F (fusion)

glycoprotein (primer sequence ID Nos. 97 and 98, Tables 2 and 4) followed by DNA sequencing or digestion with restriction enzymes (ApaL I and Avr II). Table 6 summarizes the size of the F fragments obtained for different hMPV isolates following enzymatic digestion and the corresponding genotype (see also Figure 2 for an example of the band pattern following electrophoresis on an agarose gel). Alternative typing methods based on the use of type-specific hMPV F primers and/or probes as well as DNA sequencing of amplified products are also within the scope of this invention. hMPV subtyping using similar methodologies for the other surface glycoprotein (i.e. the G gene) may also be used.

A method for detection of a plurality of potential hMPV strains having different genotypes may be conducted in separate reactions and physical enclosures, one type at the time. Alternatively, it could be conducted simultaneously for different types in separate physical enclosures, or in the same physical enclosures. In the latter scenario a multiplex PCR reaction could be conducted which would require that the oligonucleotides are all capable of annealing with a target region under common conditions. Since many probes or primers are specific for a determined genotype, typing an hMPV strain is under the scope of the present invention. When a mixture of oligonucleotides annealing together with more than one type of hMPV is used in a single physical enclosure or container, different detection labels would be used to distinguish one type from another.

The diagnostic kits, primers and/or probes mentioned above can be used to detect and/or identify hMPV, whether said diagnostic kits, primers and probes are used for *In vitro* or *in situ* applications. The said samples may include but are not limited to: any clinical sample, any environmental sample, any viral culture, any tissue, or any cell line.

It is also an object of the present invention that said diagnostic kits, primers and/or probes can be used alone or in combination with any other assay suitable to detect and/or identify other microorganisms (i.e. viruses, bacteria, fungi, and parasites) including but not limited to: any assay based on nucleic acids detection, any

immunoassay, any enzymatic assay, any biochemical assay, any lysotypic assay, any serological assay, any culture on specific cell lines, and any infectivity assay on animals.

In the methods and kits described herein below, the oligonucleotide probes and amplification primers have been derived from larger sequences (i.e. DNA fragments of at least 100 base pairs). All DNA sequences have been obtained either from our proprietary sequences (Table 1) or from SEQ ID No. 1 from public databases.

It is clear to the individual skilled in the art that oligonucleotide sequences other than those described in the present invention and which are appropriate for detection and/or identification of hMPV may also be derived from the proprietary fragment sequences or selected public database sequences. For example, the oligonucleotide primers or probes may be shorter but of a length of at least 10 nucleotides or longer than the ones chosen; they may also be selected anywhere else in the proprietary DNA fragments or in the sequences selected from public databases; they may also be variants of the same oligonucleotide. If the target DNA or a variant thereof hybridizes to a given oligonucleotide, or if the target DNA or a variant thereof can be amplified by a given oligonucleotide PCR primer pair, the converse is also true : a given target DNA may hybridize to a variant oligonucleotide probe or be amplified by a variant oligonucleotide PCR primer. Alternatively, the oligonucleotides may be designed from said DNA fragment sequences for use in amplification methods other than PCR. Consequently, the core of this invention is the detection and/or identification of hMPV by targeting DNA sequences which are used as a source of specific and ubiquitous oligonucleotide probes and/or amplification primers. Although the selection and evaluation of oligonucleotides suitable for diagnostic purposes require much effort, it is quite possible for the individual skilled in the art to derive, from the selected DNA fragments, oligonucleotides other than the ones listed in Tables 2 and 3 which are suitable for diagnostic purposes. When a proprietary fragment or a public database sequence is selected for its specificity and ubiquity (i.e. ability to detect all

hMPV strains), it increases the probability that subsets thereof will also be specific and ubiquitous.

The proprietary DNA fragments have been obtained as a repertory of sequences created by amplifying hMPV nucleic acids with proprietary primers. These primers and the repertory of nucleic acids as well as the repertory of nucleotide sequences are further objects of this invention (Tables 1-3).

As part of the design rules, all oligonucleotides were evaluated for their suitability for hybridization or PCR amplification by computer analysis using standard programs (i.e. the GCG Wisconsin package programs, the primer analysis software Oligo™ 6 and MFOLD 3.0). The potential suitability of the PCR primer pairs was also evaluated prior to their synthesis by verifying the absence of unwanted features such as long stretches of the same nucleotide and a high proportion of G or C residues at the 3' end (Persing, 1993, Diagnostic Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, DC. 88-109). Oligonucleotide amplification primers were synthesized using an automated DNA synthesizer (Applied Biosystems).

The oligonucleotide sequence of primers or probes may be derived from either strand of the duplex DNA. The primers or probes may consist of the bases A, G, C, or T or analogs and they may be degenerated at one or more chosen nucleotide position(s) (Nichols et al., 1994, Nature. 369:492-493). Primers and probes may also consist of nucleotide analogs such as Locked Nucleic Acids (LNA) (Koskhin et al., 1998, Tetrahedron Lett. 54:3607-3630), and Peptide Nucleic Acids (PNA) (Egholm et al., 1993, Nature. 365:566-568). The primers or probes may be of any suitable length and may be selected anywhere within the DNA sequences from proprietary fragments, or from selected database sequences which are suitable for the detection of hMPV.

Variants for a given target microbial gene are naturally occurring and are attributable to sequence variation within that gene. For example, different strains of the same viral

species may have a single or more nucleotide variation(s) at the oligonucleotide hybridization site. The person skilled in the art is well aware of the existence of variant nucleic acids and/or sequences for a specific gene and that the frequency of sequence variations depends on the selective pressure during evolution on a given gene product. The detection of a variant sequence for a region between two PCR primers may be demonstrated by sequencing the amplification product. In order to show the presence of sequence variations at the primer hybridization site, one has to amplify a larger DNA target with PCR primers outside that hybridization site. Sequencing of this larger fragment will allow the detection of sequence variation at this primer hybridization site. A similar strategy may be applied to show variations at the hybridization site of a probe. Insofar as the divergence of the target nucleic acids and/or sequences or a part thereof does not affect significantly the sensitivity and/or specificity and/or ubiquity of the amplification primers or probes, variant microbial DNA is under the scope of this invention. Variants of the selected primers or probes may also be used to amplify or hybridize to a variant target DNA.

In an other aspect of the invention there is also provided newly isolated human metapneumoviruses deposited with International Depository Authority of Canada on December 11 2003 and bearing IDAC numbers 111203-01 and 111203-02 (also referred herein as CAN 97-83 and CAN 98-75 respectively).

In a further aspect of the invention the diagnostic method is designed at performing simultaneous detection of nucleic acids from a variety of respiratory viruses using specific primers and/or probes. The method is aimed at detecting and/or quantifying conserved viral sequences from respiratory viruses for diagnostic purposes. Target nucleotide sequences were selected public databases and from viral strain sequences obtained in the inventor's laboratory.

The specific respiratory viral pathogens targeted by the diagnostic assays include all genotypes of influenza A and B viruses, hRSV, hMPV, PIV, adenoviruses, rhinoviruses, and enteroviruses. Other less common (coronaviruses, reoviruses) and

yet to discover respiratory viruses are also targets under the scope of the present invention.

The specific combination of oligonucleotides (primers and/or probes) in a multiplex format is also another object of this invention.

Composition of matters such as diagnostic kits comprising amplification primers and/or probes for the detection of respiratory viruses are also objects of the present invention.

Amplification of viral sequences

In a preferred embodiment, a PCR protocol is used for nucleic acid amplification. Multiplex assay, aimed at amplifying a plurality of respiratory viruses in the same physical enclosure, is an object of the present invention.

We aim at developing DNA-based tests or diagnostic kits to detect and identify most respiratory viruses in a limited number of amplification reactions using specific primers (Table 10) and/or probes (Table 11). Although some of the primer sequences used for amplification of the various respiratory viruses, with the exception of hMPV (SEQ. ID. Nos. 95 and 96 or 112 and 114), PIV-1 (SEQ. ID. Nos. 147 and 148), PIV-3 (SEQ. ID. Nos. 151 and 152), and adenovirus (SEQ. ID. Nos. 153), have been described previously by others (Fouchier et al., 2000, J. Clin. Microbiol. 38:4096-4101 for influenza A virus / SEQ. ID. Nos.139 and 140; Li et al., 2001, J. Clin. Microbiol. 39:696-704 for influenza B virus / SEQ. ID. Nos. 141 and 142; Mazulli et al., 1999. J. Infect. Dis. 180:1686-1689 for hRSV / SEQ. ID. Nos. 143 and 144; Osiowy et al., 1998, J. Clin. Microbiol.. 36: 3149-3154 for PIV-1, PIV-3, and adenovirus / SEQ. ID. Nos. 145 and 146, 149 and 150, and 154 and 155, respectively; Atmar et al., 1993, J. Clin. Microbiol. 31: 2544-2546 for rhinovirus and enterovirus / SEQ. ID. Nos. 160 and 161) and that one influenza A primer (SEQ. ID. No. 139) is

almost identical to the one described in another patent (U.S. patent number 6,015,664), the object of the present invention is to combine many sets of primers and/or probes in the same physical enclosure (multiplex format).

Briefly, viral RNA was extracted from 200 µl of naso-pharyngeal aspirates or other respiratory tract samples using the QIAamp Viral RNA Mini kit (QIAGEN, Mississauga, ON, Canada). Complementary DNA (cDNA) was then synthesized using 10 µl of the RNA preparation, 0.75 µM of random hexamer primers (Amersham Pharmacia Biotech, Baie d'Urfé, QC, Canada), 300 copies of an internal control template, and the Omniscript Reverse Transcriptase Kit (QIAGEN, Mississauga, ON, Canada) following the manufacturer's instructions. The internal control template consisted of a 554 or 558 bp (depending on the multiplex assay) transcribed region of an herpes simplex virus type 2 DNA polymerase region flanked by either influenza B or rhinovirus/enterovirus complementary primer sequences (depending on the multiplex assay) cloned in the pDrive plasmid (QIAGEN).

For DNA amplification by the widely used PCR method, primer pairs were mostly derived from public database sequences (Table 10). During DNA amplification by PCR, two oligonucleotide primers binding respectively to each strand of the heat-denatured target DNA from the viral genome are used to amplify exponentially *in vitro* the target DNA by successive thermal cycles allowing denaturation of the DNA, annealing of the primers and synthesis of new targets at each cycle (Persing, 1993, Diagnosis Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C., 88-109).

In a preferred embodiment, cDNA was amplified using a real-time PCR procedure with the LC Faststart DNA Master SYBR Green 1 Kit (Roche Diagnostics, Laval, QC, Canada) or the SYBR Green TAQ ReadyMix for quantitative PCR capillary formulation (Sigma-Aldrich, Oakville, ON) in a LightCycler instrument (Roche Diagnostics). Each reaction had a total volume of 20 µl including 2 µl of cDNA and 18 µl of a reaction mixture containing 4 mM $MgCl_2$, 2 µl of LC Faststart DNA SYBR

Green 1 Master Mix or the SYBR Green TAQ ReadyMix for quantitative PCR capillary formulation, 3% DMSO, and 0.3-1.0 μM of each viral primer (see Tables 10, 12, and 13 for primer sequence ID NOs., length of amplicons, and specific combination of primers, respectively). Cycling conditions typically included an initial denaturation step of 10 min at 94°C, followed by 50 cycles of 15 s at 94°C, 5 s at 58°C, and 25 s at 72°C. However, the cycling conditions could vary slightly according to the multiplex PCR assay.

In a preferred embodiment, two real-time multiplex PCR assays were used to amplify and detect the panel of common respiratory viruses (Table 13). The respiratory-1 multiplex assay combines 4 sets of primers for amplification of influenza A and B viruses, hRSV, hMPV, and an internal control template. The respiratory-2 multiplex assay comprises 4 other sets of primers for amplification of PIV-1, PIV-3, adenoviruses, rhinoviruses/enteroviruses, and an internal control template. The use of any other combinations of the same or similar primer sequences in two or more than two multiplex assays for detection of common respiratory viral pathogens is also under the scope of this invention.

The person skilled in the art of nucleic acid amplification knows the existence of other rapid amplification procedures such as ligase chain reaction (LCR), reverse transcriptase PCR (RT-PCR), solid phase PCR, transcription-mediated amplification (TMA), self-sustained sequence replication (3SR), nucleic acid sequence-based amplification (NASBA), strand displacement amplification (SDA), branched DNA (bDNA), cycling probe technology (CPT), solid phase amplification (SPA), rolling circle amplification technology (RCA), solid phase RCA, anchored SDA, and nuclease dependent signal amplification (NDSA) (Persing, 1993, Diagnosis Molecular Microbiology: Principles and Applications, American Society for Microbiology, Washington, D.C., 88-109; Lee, 1997, Nucleic Acid Amplification Technologies: Application to Disease Diagnosis, Eaton Publishing, Boston, MA., 61-125; Westin et al., 2000, Nat. Biotechnol.18:199-204). The scope of this invention is not limited to the use of amplification by PCR, but rather includes the use of any nucleic acid

amplification method or any other procedure which may be used to increase the sensitivity and/or the rapidity of nucleic acid-based diagnostic tests. The scope of the present invention also covers the use of any nucleic acids amplification and detection technology including real-time or post-amplification detection technologies, any amplification technology combined with detection, any hybridization nucleic acid chips or array technologies, any amplification chips or combination of amplification and hybridization chip technologies. Detection and identification by any nucleotide sequencing method is also under the scope of the present invention.

Detection of amplification products

Classically, the detection of PCR amplification products is performed by standard ethidium bromide-stained agarose gel electrophoresis. Using this approach, the amplicons may be discriminated based on their size. In the present invention, the length of the different amplicons varies from 84 to 929 bp (Table 12). It is however clear that other detection methods of amplified products may be faster, more sensitive, and more practical. Amplicon detection may also be performed by solid support or liquid hybridization using strain-specific, internal DNA probes hybridizing to an amplification product. Such probes may be generated from sequences designed to specifically hybridize to DNA amplification products which are objects of the present invention. Amplicons can be also characterized by DNA sequencing. Alternatively, a rapid detection method developed with real-time PCR assays consist of determining the specific melting temperature of amplicons at the end of the amplification reaction.

The detection of viral amplicons was first characterized using the melting curve analysis feature of a real-time PCR instrument. Using this procedure, the different viral targets amplified in each of the multiplex PCR assays could by differentiated by their specific melting temperature (Table 13 and Fig. 7 and 8). Alternatively, specific fluorescent probes (Table 11) for each viral target can be used for real-time detection and quantification of amplicons in a single amplification/detection assay. Real-time quantification of viral products can be performed through a variety of fluorescence-

based technologies such as adjacent probes, Taqman probes, and molecular beacons. Alternatively, these probes may be used in a separate post-amplification step.

The diagnostic kits, primers and probes mentioned above can be used to detect and/or identify respiratory viruses, whether said diagnostic kits, primers and/or probes are used for *in vitro* or *in situ* applications. The said samples may include but are not limited to: any clinical sample, any environmental sample, any viral culture, any tissue, and any cell line.

It is also an object of the present invention that said diagnostic kits, primers and/or probes can be used alone or in combination with any other assay suitable to detect and/or identify and /or quantify microorganisms, including but not limited to: any assay based on nucleic acids detection, any immunoassay, any enzymatic assay, any biochemical assay, any serological assay, any culture on specific cell lines, and any infectivity assay on animals.

In the methods and kits described herein, the oligonucleotide probes and amplification primers have been derived from larger sequences (i.e. DNA fragments of at least 100 base pairs). It is clear to the individual skilled in the art that oligonucleotide sequences other than those described in the present invention and which are appropriate for detection and/or identification of any respiratory viruses may also be derived from those selected in the present invention. For example, the oligonucleotide primers or probes may be shorter but of a length of at least 10 nucleotides or longer than the ones chosen; they may also be variants of the same oligonucleotide. If the target DNA or a variant thereof hybridizes to a given oligonucleotide, or if the target DNA or a variant thereof can be amplified by a given oligonucleotide PCR primer pair, the converse is also true; a given target DNA may hybridize to a variant oligonucleotide probe or be amplified by variant oligonucleotide PCR primers. Alternatively, the oligonucleotides may be designed for use in amplification methods other than PCR. Consequently, one aspect of the present invention is the simultaneous detection and/or identification of various respiratory viruses by targeting nucleic acid sequences which are used as a

source of specific and ubiquitous oligonucleotide probes and/or amplification primers. Although the selection and evaluation of oligonucleotides suitable for diagnostic purposes require much effort, it is quite possible for the individual skilled in the art to derive, from the selected oligonucleotides (listed in Tables 10 and 11), other oligonucleotides which are suitable for diagnostic purposes.

Thus, the specific primers and/or probes selected for amplification and detection of respiratory viruses and their specific combination in multiplex assays are objects of this invention (Tables 10-13).

EXAMPLES:

Example 1: Evaluation of different primers for hMPV detection

An evaluation of the sensitivity of different hMPV primer sets in a real-time PCR assay was performed using 20 positive hMPV cultures grown in LLC-MK2 cells and 10 naso-pharyngeal aspirate specimens. These samples were collected in the Québec City area (QC, Canada) over a period of 3 years (2000-2002). Viral RNA was prealably extracted from 200 µl of viral culture supernatants or naso-pharyngeal aspirates using the QIAamp Viral RNA Mini Kit (QIAGEN). Complementary DNA was synthesized using 10 µl of eluted RNA, 0.75 µM of a specific hMPV primer (Table 2, sequences ID Nos. 91, 97, 100, 102, 112), and the Omniscript Reverse Transcriptase Kit (QIAGEN, Mississauga, ON, Canada) following the manufacturer's recommendations. Complementary DNA was amplified using a real-time PCR procedure with the LC Faststart DNA Master SYBR Green 1 Kit (Roche Diagnostics, Laval, QC, Canada) in a LightCycler instrument (Roche Diagnostics). Each reaction had a total volume of 20 µl including 2 µl of RT mixture and 18 µl of a reaction mixture containing 4 mM $MgCl_2$, 2 µl of Faststart DNA SYBR Green Master Mix, 3% DMSO, and 0.5 µM of each hMPV primer (Table 4: sequence ID Nos.: 92 and 94; 100 and 101; 97 and 98; 102 and 103; and 112 and 113, for the nucleoprotein (N),

matrix (M), fusion (F), phosphoprotein (P), and polymerase (L) genes respectively). Cycling conditions typically included an initial denaturation step of 10 min. at 94°C, followed by 50 cycles of 15 s at 94°C, 5 s at 54°C, and 30 s at 72°C. However, the cycling conditions could slightly vary according to the hMPV gene to amplify. Detection of hMPV amplicons was characterized using the melting curve analysis feature of a real-time PCR instrument. Briefly, following the last amplification cycle on a LightCycler instrument, the internal temperature was rapidly increased to 94°C then decreased to 60°C for 30 seconds, followed by slowly increasing to 94°C at a rate of 0.1°C per second, with continuous fluorescence lecture.

Using this procedure, the specific melting temperature obtained with the hMPV amplicons produced by the primers specific for target genes coding for N, M, F, P and L proteins was found to be 82.63°C ± 0.87°C, 82.75°C ± 0.80°C, 83.29°C ± 0.45°C, 80.48°C ± 0.46°C, and 77.99 ± 0.33°C, respectively. As shown in Table 5, a real-time PCR assay using primers complementary to the N gene successfully amplified RNA from all 20 cultures and 10 clinical specimens. In contrast, hMPV primers specific for the M, F, P, and L genes had sensitivities of only 70%, 63.3%, 50%, and 89.7% respectively. Thus, primers aimed at amplifying the hMPV N gene appear to target more conserved regions of the hMPV genome and, consequently, were selected for further detailed evaluation (see example 2).

Example 2: Validation of a real-time PCR assay for the hMPV nucleoprotein (N) gene in a pediatric population

An hMPV N plasmid was constructed by subcloning the amplified hMPV N region in the plasmid pDrive (QIAGEN, Mississauga, ON, Canada). The new plasmid was transcribed using the RNA Transcription Kit (Stratagene, Vancouver, BC, Canada) for sensitivity analysis. Using the previously described RT procedure (N primer, sequence ID No. 91) and real-time PCR protocol (N primers, sequence ID Nos. 92 and 94) reported in example 1, the sensitivity of the assay was estimated at 100 copies per reaction with a specific melting temperature of 82 °C. The assay was found to be

specific with no amplification signal observed when viral cultures positive for the human respiratory syncytial virus, the parainfluenza viruses, the influenza viruses A and B, and the adenoviruses were tested. The real-time PCR assay for the hMPV N gene was subsequently validated in a prospective case-control study in children below the age of 3 years hospitalized at Laval University Hospital Center (CHUQ-CHUL) in Québec City, Canada, during the winter season of 2001-02. Cases consisted of children hospitalized for a respiratory syndrome whereas controls consisted of children hospitalized during the same period for a surgery with no respiratory symptoms. At admission, a nasopharyngeal aspirate was collected for viral antigen tests, viral cultures, and RT-PCR studies for influenza A and B viruses, the human respiratory syncytial virus (hRSV), and the hMPV N gene.

No virus was detected in any of the samples from the 51 control children. Among the 208 cases tested by real-time PCR for hMPV, hRSV, influenza A and B viruses, the positivity rates were 5.8%, 51.0%, 21.6%, and 0%, respectively. Viral culture was positive for hMPV in only 2 cases whereas the real-time hMPV PCR assay for the N gene was positive for 12 cases including the 2 positive by viral culture. All positive hMPV PCR results for the N gene were confirmed by a second PCR assay targeting the hMPV fusion (F) gene and by the use of DNA sequencing which indicated the presence of multiple hMPV strains clustering in the two previously-reported F genotypes. Thus, the real-time PCR assay describes in this invention appears to be much more sensitive than conventional viral culture for the detection of hMPV in clinical samples.

Example 3: Typing of hMPV clinical strains using restriction enzyme analysis

We designed a rapid molecular typing method for hMPV epidemiological studies based on amplification of a portion of the fusion (F) gene which is then digested with specific restriction enzymes. Briefly, a 759-bp fragment of the hMPV F gene was amplified by a real-time PCR procedure as described in example 1 using specific primers (sequence ID Nos. 97 and 98). After PCR amplification, two aliquots (10 uL) of the PCR product were digested during 2 hours at 37°C with 10 units of ApaL I and 4 units of Avr II (New England Biolabs, Mississauga, ON, Canada) according to the manufacturer's recommendations. Digested products were then loaded on an 1% agarose gel for electrophoresis.

Table 6 summarizes the size of the F fragments obtained for different hMPV isolates following enzymatic digestion and the corresponding genotype as defined by DNA sequencing and phylogenetic studies. In brief, all hMPV isolates belonging to the genotype F-I were digested by ApaL I (fragments of 532 and 227 bp) but not by Avr II whereas those belonging to the genotype F-II were digested by Avr II (fragments of 447 and 312 bp) but not by ApaL I (see Figure 2 for an example of the band pattern following electrophoresis on an agarose gel).

Example 4 : A prospective case-control study for defining the role of respiratory viruses in children hospitalized for acute respiratory tract infections

Summary

The human metapneumovirus (hMPV) is a new paramyxovirus recently isolated from young children with respiratory syndromes. The objectives of this study were to estimate the relative contribution of hMPV in children's hospitalization for acute respiratory tract infections (ARTI) and to define its clinical presentation and seasonal pattern.

We evaluated the percentage of hospitalizations for ARTI in children aged 0-3 years attributate to hMPV and other respiratory viruses in a prospective case-control study during the winter/spring seasons of 2002. We used multiplex real-time PCR assays and other conventional diagnostic methods to detect hMPV, the human respiratory syncytial virus (hRSV), and the influenza viruses in nasopharyngeal aspirates of children.

HMPV was detected in 12 (5.8%) of the 208 children hospitalized for ARTI as compared to 118 (56.7%) for hRSV and 49 (23.6%) for influenza A. In contrast, none of the 51 controls harbored any of the respiratory viruses. Most hRSV and influenza infections occurred in January and February whereas the peak hMPV activity was in March and April. The peak age for hospitalizations for hRSV and hMPV infections was < 3 months and 3-5 months, respectively. Diagnoses of bronchiolitis and pneumonitis were made in respectively 67% and 17% of hMPV-infected children compared to 84% and 25% for those with hRSV infection. None of the hMPV-infected children was admitted to the intensive care unit compared to 15% for those with hRSV or influenza A infections.

HMPV causes significant morbidity in young children and its clinical presentation is similar although less severe than that of hRSV.

Introduction

The human metapneumovirus (hMPV) is the first human member of the new *Metapneumovirus* genus within the *Paramyxoviridae* family (van den Hoogen BG, de Jong JC, Groen J, et al.. Nat Med 2001;7:719-24; van den Hoogen BG et al. Virology 2002;295:119-32.) The human respiratory syncytial virus (hRSV) belongs to a separate genus within the same family (Taxonomy V. Seventh Report of the International Committee on Taxonomy of Viruses. Academic, San Diego, 2000.).

HMPV has been recently identified in nasopharyngeal aspirates of children and adults suffering from a range of respiratory tract infections in various parts of the world (van

den Hoogen BG, de Jong JC, Groen J, et al. Nat Med 2001;7:719-24; Nissen MD, et al. Med J Aust 2002;176:188; Peret TC, Boivin G, Li Y, et al. J Infect Dis 2002;185:1660-3; Stockton J, et al. Emerg Infect Dis 2002;8:897-901). The clinical syndrome of the infected children encompasses mild respiratory problems to bronchiolitis and pneumonitis (van den Hoogen BG, de Jong JC, Groen J, et al. Nat Med 2001;7:719-24; Pelletier G, et al. Emerg Infect Dis 2002;8:976-8; Boivin G, Abed Y, Pelletier G, et al. J Infect Dis 2002;186:1330-4).

Using reverse-transcription polymerase chain reaction (RT-PCR), the rate of hMPV detection in nasopharyngeal aspirates (NPA) of pediatric patients with unexplained ARTI varied between 1.5 and 10% (van den Hoogen BG, de Jong JC, Groen J, et al. Nat Med 2001;7:719-24; Nissen MD, et al. Med J Aust 2002;176:188). These retrospective studies are small, excluded patients who tested positive for other viruses, only superficially describe the clinical features of the disease and lack morbidity/mortality data. In absence of a control group, these studies cannot differentiate if hMPV is a colonizing or a pathogenic virus. More recently, Stockton et al. identified hMPV RNA in 2.2% of 405 specimens from patients with influenza-like illnesses who consulted general practitioners in England although few swabs were collected from children < 5 years (Stockton J, et al. Emerg Infect Dis 2002;8:897-901).

The objectives of this study were to estimate the relative contribution of hMPV in children's hospitalization for acute respiratory tract infections (ARTI) and to define its clinical presentation and seasonal pattern.

Materials and Methods

Study design. Participants were children aged ≤ 3 years hospitalized between December 15, 2001 and April 20, 2002 at Laval University Hospital Center (CHUQ-CHUL) in Québec City, Canada. Cases were admitted for an ARTI (bronchiolitis, pneumonitis, laryngotracheobronchitis, etc...) whereas controls were hospitalized for an elective surgery and had no respiratory symptoms or fever. Children hospitalized twice were counted as two cases. At admission, after signed consent was obtained from their parents, all children had an NPA (1-2 ml) collected and tested for hRSV antigen (TestPack, Abbott Laboratories, Abbott Park, IL). The specimen was frozen at -80°C until subsequent RT-PCR studies. Additionally, viral cultures, direct immunofluorescence staining, and microbiological testing were performed according to the treating physician. A standardized questionnaire was completed at admission by a single research nurse with the parents. At the end of the hospitalization, the children's charts were reviewed to collect clinical and laboratory data. The study was approved by the CHUQ-CHUL research ethics board.

RNA extraction and cDNA synthesis. Viral RNA was extracted from 200 µl of NPA specimens using the QIAamp viral RNA Mini Kit (QIAGEN, Mississauga, ON, Canada). Complementary cDNA was synthesized using 10 µl of eluted RNA and the Omniscript Reverse Transcriptase kit (QIAGEN). Random hexamer primers (Amersham Pharmacia Biotech, Baie d'Urfé, QC, Canada) were used in the RT step of the multiplex respiratory PCR assay whereas a specific primer (5'-TGGGACAAGTGAAAATGTC-3') serves to synthesize hMPV cDNA in a separate PCR assay. An internal control (300 copies) consisting of a 534-bp transcribed region of the pDrive plasmid (QIAGEN) flanked by influenza B complementary primer sequences (see below) was spiked in the RT reaction of the multiplex respiratory PCR assay to verify the presence of PCR inhibitors.

RT-PCR assays and phylogenetic studies. The multiplex respiratory PCR assay was designed to amplify conserved regions of the influenza A (SEQ. ID Nos.139-140) (Fouchier RA, et al. J Clin Microbiol 2000;38:4096-101) and B (SEQ. ID Nos. 141-

142) (Li J, Chen S, Evans DH. J Clin Microbiol 2001;39:696-704) matrix genes as well as the fusion gene of hRSV (SEQ. ID Nos. 143-144) (Mazzulli T, Peret TC, McGeer A, et al. J Infect Dis 1999;180:1686-9).

Complementary DNA was amplified using a real-time PCR procedure with the LC Faststart DNA Master SYBR Green 1 Kit (Roche Diagnostics, Laval, Québec, Canada) in a LightCycler instrument (Roche Diagnostics). Each reaction had a total volume of 20 µl including 2 µl of cDNA and 18 µl of a reaction mixture containing 2.4 mM $MgCl_2$, 2 µl of Faststart DNA SYBR Green Master Mix, 3% DMSO, 0.3 mM each of influenza A and B primers, and 0.8 mM of hRSV primers. The thermal cycling incubations consisted of an initial denaturation step at 94°C for 10 min, followed by 50 cycles of 15 s at 94°, 5 s at 58°C, and 25 s at 72°C. At the end of each cycle, the fluorescent signal was measured at a wavelength of 530 nM using the LightCycler Fluorimeter. The melting curve analysis program of the LightCycler was used to identify specific PCR products of influenza A (Tm: 85°C), influenza B (Tm: 83°C), hRSV (Tm: 79°C), and the internal control (Tm: 92°C). The sensitivity of the multiplex respiratory PCR assay was estimated at 50 copies for each viral target when mixed with 300 copies of the internal control.

A specific PCR assay was designed for amplification of the hMPV Nucleoprotein (N) gene in the LightCycler instrument. The sequences of the forward (MPV-NCS) and reverse (MPV-NCA) primers were respectively 5'-GAGTCTCAGTACACAATTAA-3' (SEQ ID NO 92) and 5'-GCATTTCCGAGAACAACAC-3' (SEQ ID NO 94). The specific cDNA was amplified using conditions similar to those of the multiplex respiratory PCR assay except that 0.5 mM of each primer was added to the master mix. Cycling conditions included an initial denaturation step of 10 min at 94°C, followed by 50 cycles of 15 s at 94°C, 5 s at 54°C, and 30 s at 72°C. The melting temperature of the amplified hMPV N fragment was 82°C and the sensitivity of the PCR assay was 100 copies. For phylogenetic studies, nucleotide sequences were determined from amplified hMPV F (fusion) gene products then analyzed using the

neighbor joining algorithm and Kimura-2 parameters. (Boivin G, Abed Y, Pelletier G, et al. J Infect Dis 2002;186:1330-4).

hMPV in the general population. To further assess the seasonal distribution, affected age groups and frequency of hMPV, we compared data from the study to those of the general population derived from the viral cultures found positive for hMPV in our regional virology diagnostic laboratory, the only one performing viral cultures for the Québec City area (population approximately 600 000). Respiratory specimens were inoculated in 96-well plates containing ten cell lines (MDCK, LLC-MK2, Hep-2, human foreskin fibroblast, Vero, mink lung, A-549, rhabdomyosarcoma, 293, and HT-29) then incubated for a period of 21 days. A positive cytopathic effect was confirmed by immunofluorescence testing using monoclonal antibodies or by RT-PCR (hMPV).

Statistical analyses. Proportions were compared by the χ^2 test.

Results

The study population included a total of 208 hospitalized cases with ARTI (including 8 children who were admitted twice) and 51 children who served as controls.

Description of the study population and viral etiologies. The age distribution of children with ARTI is presented in Fig. 3: 56% (116) were male and 44% (92) were female. Hospitalization was the highest in infants ≤ 3 months of age and steadily decreased thereafter. Most (90%) children had no underlying medical conditions at admission.

All 208 cases had an NPA. The mean delay between the onset of symptoms and collection of NPA was 6 days (median 4 days). This delay did not differ for the different viruses detected. All 208 cases were tested by PCR, 204 had an hRSV

antigen detection test, 100 had a direct examination procedure for other viruses, and 100 had a viral culture. At least one respiratory virus was detected in 164 (79%) cases whereas none was detected in 44. Twelve children (5.8%) were positive for hMPV, 118 (56.7%) for hRSV, 49 (23.6%) for influenza A, and none for influenza B (Table 7). There was no PCR testing for adenoviruses and PIV type 2 but these two viruses were found in 6% and 2%, respectively, of the 100 viral cultures or antigenic assays. Single virus infections occurred in 141 (86%) of the 164 positive cases and mixed infection was present in 23 (14%). Two of the 12 hMPV infections were mixed (hMPV-influenza and hMPV-hRSV). The other combinations were hRSV-influenza A (18 cases), hRSV-adenovirus (2), and influenza A-adenovirus (1).

Among the 208 cases tested by PCR for hMPV, hRSV, influenza A and B viruses, the positivity rates were 5.8%, 51.0%, 21.6%, and 0%, respectively (Table 7). In addition, 16 other cases had one of the four latter respiratory viruses identified only by culture (3 influenza A and one hRSV), only by an antigen detection test (10 hRSV and one influenza A) or by both culture and antigen detection test (1 hRSV). Among the 8 children hospitalized twice, none had the same viral infection at both admissions. The combinations observed were hMPV – hRSV (2), hMPV – no virus (1), hRSV – no virus (2), hRSV – influenza (2), no virus – no virus (1).

The biweekly distribution of cases with respiratory tract viruses is shown in Fig. 4. hRSV and influenza A infections occurred predominantly between January and March, whereas hMPV infections occurred mostly in March and April. The proportion of children with virologically-confirmed respiratory tract infection decreased after February.

Clinical features of cases. The distribution of the age distribution by different viral infections is shown in Fig. 3. While the peak age for hMPV infection was 3-5 months, it was before 3 months for hRSV infection. For influenza A, it spread evenly during the first year of life. The peak age for mixed infection was 6-11 months and decreased thereafter. Gender was distributed evenly with each virus group but more males

(70%) had mixed viral infections. The majority (75% with hMPV, 93% with hRSV, 90% with influenza A virus infection) of the children in different etiological groups had no medical underlying conditions. Three (25%) children with hMPV infection had a cardiac disorder, one having concomitant cardiac, pulmonary, and neurological problems.

Signs and symptoms recorded with the different respiratory viruses were generally similar except for the vomiting which occurs more frequently with hMPV infections ($p = 0.05$) (Table 8). The mean and median duration of hospitalization was similar for hMPV, hRSV and influenza A viruses being respectively 5.5 and 4.5, 5.8 and 5.0, and 4.9 and 4.0 days. Of note, one third of hMPV-infected children were hospitalized more than 7 days. None of the children with hMPV infection was admitted to the intensive care unit (ICU) contrasting with 15% and 16% of children with hRSV and influenza A, respectively. There was no death as part of this study. The duration of the hospitalization in children with no detectable virus was shorter than that in children with single or mixed infection (Kruskal-Wallis Test, $p < .0001$). Two-thirds of the children were given antibiotics during their hospitalization, independently of the etiology of their respiratory disease.

At hospital discharge, a final diagnosis of bronchiolitis was given to 67% of children with hMPV, 84% with hRSV, and 51% with influenza A ($p < 0.001$) (Table 9). Otitis occurred in about half of children with hMPV, hRSV, and influenza A infections. Pneumonitis was less frequently diagnosed in children with hMPV compared to those with hRSV or influenza A (17%, 25%, and 37%, $p=0.22$). Final diagnoses were similar with single and mixed infections.

Controls. There were 51 children in the control group, 29 (56.9%) males and 22 (43.1%) females. The age distribution of the controls was similar to that of the cases (Fig. 3) as was the period of hospital admission (Fig. 4). None of the controls had a virus detected by PCR.

hMPV in the general population. The regional virology laboratory received 1505 respiratory specimens for viral culture between January 1 and June 30, 2002. In total, 36 (including two study participants) or 2.9% were positive for hMPV: 24 (66.6%) in children < 2 years of age, 5 in those 2 to 4 years, 4 in adults 30-49 years, and 3 in those 70 years or older. Most cases (81%) were isolated over a two-month period (from March 23 to May 18) (Fig. 5). When comparing the seasonal distribution of hMPV in hospitalized children (study population) and in the general population, it appears that the study in hospitalized children did not cover the entire hMPV season as it was stopped just after the peak time of hMPV transmission (April 6-20).

Phylogenetic analyses of hMPV strains. The 12 hMPV strains isolated in the prospective study clearly clustered into two F lineages with nine strains belonging to group 1 and three to group 2 (Fig. 6). Seven of the 9 group 1 strains had identical F gene sequences. At the nucleotide level, there was 84-85% similarity between groups versus 98-100% (group 1) and 93-99% (group 2) similarity within groups. There were fewer differences at the amino acid level i.e. 94-96% similarity between groups versus 99-100% (group 1) and 98-99% (group 2) among strains within the same group. Although there was a trend for more recent strains to cluster in group 2, the correlation between genotypes and date of hospitalization was not perfect as two strains detected on the same day (No. 216 and 217) belonged to two different F lineages.

Discussion

Our prospective study has revealed important clinical and epidemiological features related to hMPV. First, our data indicate that hMPV is really a respiratory pathogen. Second, we found that it contributes to ARTI leading to children's hospitalization in proportion similar to adenoviruses or PIV but significantly less than hRSV and influenza viruses. Third, the symptoms and complications associated with hMPV were indistinguishable from those caused by hRSV. Finally, our results suggest that the epidemiology of hMPV infection in children may differ from that of hRSV and

influenza viruses both in terms of the age of first acquisition and in the timing of the seasonal epidemics.

Recent studies by our group (Pelletier G, et al. Emerg Infect Dis 2002;8:976-8; Boivin G, Abed Y, Pelletier G, et al. J Infect Dis 2002;186:1330-4) and others (van den Hoogen BG, de Jong JC, Groen J, et al Nat Med 2001;7:719-24.; Nissen MD et al. Med J Aust 2002;176:188; Stockton J, et al. Emerg Infect Dis 2002;8:897-901) had suggested that hMPV should be added to the list of human respiratory viral pathogens (Dowell SF, Anderson LJ, Gary HE, Jr., et al. J Infect Dis 1996;174:456-62; Treanor JJ. Respiratory Infections. In: Richman DD, Whitley, R.J., Hayden, F.G., ed. Clinical Virology. Vol. 1. Washington: ASM Press, 2002:7-26).

The absence of hMPV in respiratory tract of our 51 control children, the absence of other respiratory viruses in 83% of the 12 hMPV-infected children and the severity of their symptoms (bronchiolitis and/or pneumonitis) provide further evidence that hMPV is really a pathogenic respiratory virus. The absence of underlying medical conditions in 75% of the hMPV-infected children further demonstrates its pathogenicity. The use of PCR assays was particularly advantageous for hMPV because its growth in culture is fastidious and limited to a few permissive cell lines (Boivin G, Abed Y, Pelletier G, et al. J Infect Dis 2002;186:1330-4) and because rapid antigenic detection tests are currently unavailable.

Our study provides for the first an estimate of the proportion of ARTI hospitalizations attributable to hMPV in a pediatric population. During the period ranging from December 15 to April 20 2002, hMPV accounted for 5.8% of all hospitalizations for respiratory tract infections in children aged 0-3 years. This underestimates the real proportion as our hospitalization study stopped before the end of the hMPV season prevailing in the community (Fig. 5). Although we did not perform PCR testing for adenoviruses and PIV, the percentage of hospitalizations caused by hMPV seems similar to that attributable to these two viruses and much smaller than that of hRSV or influenza A.

One of the most important conclusion of our study is that hMPV disease cannot be distinguished on clinical findings from that caused by hRSV and influenza A (Tables 8 and 9) but tended to be somewhat less severe with fewer pneumonia and no admission in the ICU. Nevertheless, hMPV infection was associated with a significant clinical and economical burden as shown by a mean hospital stay of 5.5 days and one-third of hMPV-infected cases being hospitalized for > 7 days.

A small serological study from the Netherlands showed that all children over the age of 5 years had hMPV antibodies suggesting a high level of transmission.[1] Data from our study suggest that morbidity caused by hMPV is greatest in children < 2 years of age as they represented 83% (10/12) of our hospitalized cases and two-thirds of the hMPV isolates recovered in our diagnostic virology laboratory. This suggests that similar to other paramyxoviruses such as hRSV, the morbidity of hMPV in children occurs through primo-infection. In contrast to hRSV which peaked during the first three months of life, hMPV infections peaked at a slightly older age i.e. between the third and fifth month of life. Whether this difference depends on a longer persistence of maternal antibodies or a less efficient transmission mode in the case of hMPV would require additional studies.

During the 4-week period from mid-March to mid-April, hMPV infections clustered (11/12 detected) and accounted for 18.9% of all hospitalizations for ARTI in children. This contrasts with hRSV and influenza A which occurred mostly in January and February (Figure 4). Based on passive surveillance data from our regional virology laboratory, the peak time of hMPV transmission in the community occurred between April 6-20, 2002, and continued beyond the end of our study in hospitalized children (Fig. 5). Year-long active surveillance studies will be required to better assess the seasonal patterns of hMPV transmission.

As described for hRSV (Mazzulli T, Peret TC, McGeer A, et al J Infect Dis 1999;180:1686-9), several strains of hMPV circulated during a very short period of

time (one month) in our study area. The hMPV strains segregated into two F subgroups in agreement with previous studies although one strain clearly predominated accounting for 58.3% of all isolates (van den Hoogen BG, de Jong JC, Groen J, et al. Nat Med 2001;7:719-24; Peret TC, Boivin G, Li Y, et al. J Infect Dis 2002;185:1660-3; Boivin G, Abed Y, Pelletier G, et al. J Infect Dis 2002;186:1330-4).

Due to the small number of hMPV strains belonging to one of the F subgroup, we did not attempt to correlate hMPV genotype with clinical outcome. Such viral heterogeneity may allow multiple reinfections throughout life especially in elderly subjects and immunocompromised patients (Pelletier G, Dery P, Abed Y, Boivin G Emerg Infect Dis 2002;8:976-8).

In conclusion, our study supports the concept of the epidemic nature of hMPV infection and establishes its role as a significant pathogen in severe ARTI of children.

Example 5: Determination of the hMPV Load in Clinical Samples Using Quantitative Real-time PCR

An evaluation of a quantitative real-time PCR hMPV assay using a TaqMan probe was performed using naso-pharyngeal aspirate specimens collected from children and adults in Québec City during the winter seasons of 2001-02 and 2002-03. Viral RNA was first extracted from 200 µl of naso-pharyngeal aspirates using the QIAamp Viral RNA Mini Kit (QIAGEN) and eluted in a volume of 40 µl. Serial concentrations ($5X10^2$ to $1X10^9$ copies) of a transcribed hMPV plasmid were used to construct the standard curve of the assay. Complementary DNA was synthesized using 10 µl of eluted RNA from clinical samples or hMPV plasmid dilutions, 50 ng (0.75 µM) of random hexamer primers (Amersham Pharmacia Biotech), 0.5 mM of dNTPs, 1X Omniscript buffer and 4 units of the Omniscript reverse transcriptase (QIAGEN) in a final volume of 20 µl. The reverse transcription (RT) mixture was incubated during 1.5 hr at 37°C and inactivated at 70°C during 10 min. Complementary DNA was amplified by real-time PCR with a TaqMan probe for the hMPV L gene and the LC

Master Hybridization Probe Mix (Roche Diagnostics) in a LightCycler instrument (Roche Diagnostics). Each reaction had a volume of 20 µl including 2 µl of RT mixture, 4 mM MgCl2, 0.3 µM of specific hMPV L primers (sequence ID Nos. 112 and 114), 0.6 µM of a specific hMPV L probe (SEQ. ID. No. 127), and 2 µl of the LC Master Hybridization Probe Mix (Roche Diagnostics). Cycling conditions included an initial denaturation step of 30 s at 94°C, followed by 60 cycles of 5 s at 94°C, 5 s at 56°C, and 25 s at 72°C. A fluorescence lecture was taken after each elongation step (Fig. 9A). A standard curve for the PCR reaction was constructed by plotting cycle threshold values against log concentrations of each hMPV plasmid dilution. The number of hMPV copies present in clinical samples was calculated by interpolating cycle threshold values into the assay's standard curve (Fig. 9B).

Example 6: Determination of hMPV genotypes based on amplification and sequencing of the viral F and G genes.

Determination of hMPV genotypes was achieved by PCR amplification of the F or G genes followed by DNA sequencing of the viral amplified products. Different sets of primers were used for PCR amplification and DNA sequencing of the F (SEQ. ID. Nos. 97 and 98) and G (SEQ. ID. Nos. 104 and 107 or 108 and 109) genes. Alignment of viral DNA sequences was done with the CLUSTAL W software version 1.7 for Unix and phylogenetic trees were computed by maximum parsimony, distance and maximum likelihood-based criteria analysis using PAUP version 4.0.d8. Phylogenetic trees for both the F (Fig. 10A) and G (Fig. 10B) genes from Canadian and non-Canadian viral isolates revealed the existence of two hMPV groups (genotypes A and B) with two subgroups (subtypes 1 and 2) within each group.

Example 7: Comparison of viral culture and multiplex PCR assay for detection of influenza A virus.

A multiplex PCR assay (including PCR primers defined in SEQ. ID. Nos. 139, 140, 141, 142, 143, and 144) aimed at detecting influenza A and B viruses and the respiratory syncytial virus was used to assess the etiology of severe acute respiratory

tract infections (ARTI) necessitating hospitalization in children aged 0-3 years during the winter season 2001-02 at the CHUL, an university-based hospital, in Québec City (Canada). Nasopharyngeal (NPA) samples from all 208 children with ARTI were tested by multiplex PCR whereas a subset of 100 NPA specimens was inoculated on Madin Darby Canine Kidney (MDCK) cells for influenza culture. Briefly, viral RNA was prealably extracted from 200 µl of naso-pharyngeal aspirates using the QIAamp Viral RNA Mini kit (QIAGEN, Mississauga, ON, Canada). cDNA was then synthesized using 10 µl of the RNA preparation, 0.75 µM of random hexamer primers (Amersham Pharmacia Biotech, Baie d'Urfé, QC, Canada), 300 copies of an internal control template, and the Omniscript Reverse Transcriptase Kit (QIAGEN, Mississauga, ON, Canada) following the manufacturer's instructions. The internal control template consisted of a 558-bp transcribed region of an herpes simplex virus type 2 DNA polymerase region flanked by influenza B complementary primer sequences cloned into the pDrive plasmid (QIAGEN). cDNA was amplified using a real-time PCR procedure with the LC Faststart DNA Master SYBR Green 1 Kit (Roche Diagnostics, Laval, QC, Canada) in a LightCycler instrument (Roche Diagnostics). Each reaction had a total volume of 20 µl including 2 µl of complementary DNA and 18 µl of a reaction mixture containing 4 mM $MgCl_2$, 2 µl of LC Faststart DNA SYBR Green Master 1 Mix, 3% DMSO, 0.3 µM of influenza A and influenza B primers, and 0.8 µM of human respiratory syncytial virus primers (Table 10). Cycling conditions included an initial denaturation step of 10 min. at 94°C, followed by 50 cycles of 15 s at 94°C, 5 s at 58°C, and 25 s at 72°C. Detection of viral amplicons was characterized using the melting curve analysis feature of a real-time PCR instrument. Briefly, following the last amplification cycle on a LightCycler, the reaction temperature was rapidly increased to 94°C and was then decreased to 60°C for 30 seconds followed by a slow increase to 94°C at a rate of 0.1°C per second, with continuous fluorescence lecture. Using this procedure, the amplified products of influenza A, influenza B, hRSV, and the internal control could be differentiated by virtue of their specific Tm values i.e. 85, 83, 79, and 91°C, respectively (Table 13 and Fig. 11).

Influenza A virus was detected by multiplex PCR (specific Tm value of 85°C) in 45/208 (21.6%) of the samples tested whereas the culture was positive for this virus in 14/100 (14%) tested NPA samples from children. For the subset of 100 NPA specimens tested by both methods, 88 (88%) had concordant results (11 positive, 77 negative) whereas discordant results were obtained for 12 (12%) samples (3 culture$^+$/PCR$^-$, 9 culture$^-$/PCR$^+$). Assuming that all positive results (by either culture or PCR) were true positive, the sensitivity values of the culture and the multiplex PCR for influenza A virus were 60.9% and 87.0%, respectively. There was no positive result for influenza B virus by any detection method as part of this study.

Example 8: Comparison of antigen detection and multiplex PCR assay for detection of the human respiratory syncytial virus.

Same as example 1, except that the multiplex PCR assay was used to detect hRSV from NPA samples obtained from 211 children with ARTI and compared with an antigen detection test for hRSV (TestPack, Abbott Laboratories, Abbott Park, IL).

hRSV was detected by the multiplex PCR (specific Tm value of 79°C) in 109/211 (51.7%) of the tested samples whereas the hRSV antigen detection test was positive in 93/211 (44.1%) tested NPA samples from children. Concordant results were found in 183 (86.7%) samples (87 positive, 96 negative) whereas discordant results were obtained for 28 (13.3%) samples (6 antigen$^+$/PCR$^-$, 22 antigen$^-$/PCR$^+$). A second RT-PCR assay for detection of another hRSV gene (glycoprotein G) was used to resolve the discrepancies (Peret et al., 1998, J Gen Virol.79:2221-2229). After the resolution of the discrepant results, the sensitivity values of the antigen detection test and the multiplex PCR for hRSV were 80.5% and 96.5%, respectively.

Example 9: Evaluation of multiplex real time PCR assay for Influenza and human respiratory syncytial viruses.

We developed a real-time multiplex PCR assay for influenza viruses and HRSV using the melting curve analysis feature of the LightCycler instrument to rapidly distinguish viral products.

Materials and methods

Study population and antigenic test. Nasopharyngeal aspirates (NPA) were prospectively collected from children aged 0-3 years hospitalized for ARTI during the winter season 2001-02 at a single, university-based hospital, in Québec City (Canada) (Boivin, G., et al. 2003. Emerg. Infect. Dis. 9:634-640). The study was approved by the institutional review board of the Centre Hospitalier Universitaire de Québec. All samples were tested by the multiplex real-time PCR assay whereas antigen detection tests were performed on a subset of samples upon request by the treating physician. An aliquot (750 µl) of fresh NPA was first tested for the presence of HRSV and influenza A/B antigens using respectively the RSV TestPack (Abbott Laboratories, Abbott Park, IL) and the Directigen Flu A+B test (Becton Dickinson, Sparks, MD). The rest of the specimen was frozen at -80°C for a maximum of 3 months before nucleic acid extraction and PCR testing.

RNA extraction and cDNA synthesis. Viral RNA was extracted from 200 µl of NPA samples using the QIAamp Viral RNA Mini kit (QIAGEN, Missisauga, ON, Canada). Complementary DNA was then synthesized using 10 µl of the RNA preparation, 0.75 µM of random hexamer primers (Amersham Pharmacia Biotech, Bale d'Urfé, QC, Canada), and the Omniscript Reverse Transcriptase kit (QIAGEN) in presence of 300 copies of an internal control. The internal control template consisted of a 558-bp transcribed region of the herpes simplex virus type 2 DNA polymerase gene flanked by influenza B complementary primer sequences (see below) cloned into the pDrive plasmid (QIAGEN).

Real-time multiplex and conventional PCR assays. The multiplex PCR respiratory assay was designed to amplify conserved regions of the influenza A (Fouchier, R. A.,et al. . 2000.. J. Clin. Microbiol. 38:4096-4101) and B (Li, J., S. Chen, and D. H. Evans. 2001.. J. Clin. Microbiol. 39:696-704) matrix genes as well as the fusion gene of HRSV (Mazzulli, T., et al, 1999. J. Infect. Dis. 180:1686-1689). Complementary DNA was amplified using a real-time PCR procedure with the LC Faststart DNA Master SYBR Green 1 kit (Roche Diagnostics, Laval, QC, Canada) in a LightCycler instrument (Roche Diagnostics). Each reaction had a total volume of 20 µl including 2 µl of cDNA and 18 µl of a reaction mixture containing 2.4 mM $MgCl_2$, 2 µl of Faststart DNA SYBR Green 1 Master Mix, 3% DMSO, 0.3 mM of influenza A and B primers (SEQ. ID Nos. 139-140 and 141-142), and 0.8 mM of HRSV primers (SEQ. ID Nos. 143-144). Cycling conditions included an initial denaturation step of 10 min at 94°C, followed by 50 cycles of 15 s at 94°C, 5 s at 58°C, and 25 s at 72°C. At the end of each cycle, the fluorescent signal was measured at a wavelength of 530 nM using the LightCycler Fluorimeter. The melting curve analysis program of the LightCycler was used to identify specific PCR products. Briefly, following the last amplification cycle, the reaction temperature was rapidly increased to 94°C and was then decreased to 60°C for 30 s followed by a slow increase to 94°C at a rate of 0.1°C per s, with continuous fluorescence monitoring. For resolution of discrepant results between the multiplex PCR assay and antigenic tests, conventional RT-PCR assays for detection of the glycoprotein G gene of HRSV (Peret, T. C., et al. 1998. J. Gen. Virol. 79:2221-2229) and the M2 gene of influenza A viruses (Klimov, A. I., et al. . 1995. J. Infect. Dis. 172:1352-1355) were performed retrospectively.

Results

Assay's characteristics. The sensitivity of each individual real-time PCR assay and of the multiplex PCR respiratory assay was determined for each target by testing serial dilutions of transcribed plasmids containing specific viral sequences. The lower limit of detection for influenza A, influenza B, and HRSV was found to be 10, 50 and 50 copies, respectively, in individual PCR assays whereas it was 50 copies for each target (10 out of 10 times for influenza A and 9 out of 10 times for influenza B and

HRSV) in the multiplex PCR test. No amplification signal was detected in the real-time multiplex assay when testing viral DNA or RNA from adenoviruses, rhinoviruses, enteroviruses (echo 11), parainfluenza viruses 1-3, human metapneumovirus, and herpesviruses (herpes simplex virus type 1 and 2, cytomegalovirus, and varicella-zoster virus). Specific melting temperature (Tm) values were first determined by testing a set of clinical isolates and vaccine strains (11 influenza A/H1, 10 influenza A/H3, 10 influenza B, and 17 HRSV) from different years (Table 14). A representative set of results is shown in Fig.11. The mean Tm values were 85.27±0.22 , 83.47±0.46, and 79.51±0.30 °C for influenza A, influenza B, and HRSV corresponding to fragments of 245, 524, and 380 bp, respectively, after gel electrophoresis (Table 14). We found no influence of influenza A subtype (H1 and H3) and HRSV genotype (A and B) on the Tm values (Table 14). The addition of 300 copies of the internal control in the assay's master mix resulted in a specific amplification peak with a Tm value of 91°C (558 bp) in the absence of amplified viral products.

Evaluation of the multiplex PCR respiratory assay in a pediatric population. The multiplex PCR assay was evaluated using extracted RNA from 208 NPA samples collected during a prospective pediatric study aimed at assessing the role of the human metapneumovirus in hospitalized children (0-3 years) with ARTI (Boivin, G., et al. 2003. Emerg. Infect. Dis. 9:634-640). The number of positive samples by multiplex PCR for influenza A, influenza B, and HRSV was found to be 45 (21.6%), 0 (0%), and 106 (51.0%), respectively. Overall, the rate of positivity for any of the 3 viruses was 66.8% (139/208) including 5.8% (12/208) of co-infections with influenza A and HRSV. None of the samples was considered to contain PCR-inhibitory samples as verified by the amplification of the internal control in all PCR-negative samples. The ranges of Tm values for influenza A- and HRSV-positive samples were 85.10 ± 0.39 and 79.60 ± 0.42°C, respectively. All amplified products with one of the latter Tm values were confirmed as specific viral targets by visualizing the appropriate band on agarose gel. As none of the samples contained influenza B virus sequences (which correlates with the very occasional isolation of influenza B viruses in the Québec City

area during the 2001-2002 season), a clinical evaluation of the multiplex PCR assay was conducted for influenza A and HRSV only.

Evaluation of the multiplex PCR respiratory assay for detecting influenza A virus. From the 208 NPA evaluated by multiplex PCR, a subset of 172 samples were also tested for the presence of influenza antigens using the Directigen Flu A+B test (Table 15). Concordant results were obtained for 145 (84.3%) samples including 17 positive and 128 negative results by the two tests. A total of 27 (15.7%) samples showed discordant results with 25 being positive by the multiplex PCR assay only and two positive by the antigenic test only. In order to resolve the discrepant results, a second RT-PCR test targeting a conserved gene (M2) of influenza A viruses was performed as reported (Klimov, A. I., et al. . 1995. J. Infect. Dis. 172:1352-1355). As shown in Table 15, influenza A virus M2 sequences were detected by the conventional RT-PCR assay in 22 (81.5%) of the 27 discordant samples. Using the M2 RT-PCR assay as the reference test, the sensitivity, specificity, positive predictive and negative predictive values of the multiplex real-time PCR assay and the antigenic test for influenza A virus were 100%, 97.7%, 92.8%, 100% and 43.6%, 98.5%, 89.5%, 85.6%, respectively. The multiplex PCR respiratory assay was positive for HRSV in 12 (44.4%) of the 27 discordant influenza A samples with 10 of these samples showing dual HRSV/influenza A infections by the multiplex PCR assay (Table 16). The mean cycle threshold (C_T) values, which are inversely correlated with the amounts of viral RNA, were 28.1 and 33.0 for PCR-positive/antigen-positive and PCR-positive/antigen-negative samples, respectively (Table 16). These values correspond to approximately 5000 and 50 copies, respectively, when a specific influenza A plasmid was tested in the multiplex PCR assay.

Evaluation of the multiplex PCR respiratory assay for detecting HRSV. A total of 204 NPA samples could be evaluated by both the multiplex real-time PCR assay and the presence of HRSV antigens using the RSV TestPack (Table 17). Concordant results were found in 172 (84.3%) samples including 83 positive and 89 negative results by the two tests. Thirty-two (15.7%) samples had discordant results with 21

being positive by the multiplex PCR assay only and 11 by the antigenic test only. Conventional RT-PCR tests for the HRSV glycoprotein G (A and B subtypes) were performed as previously described (Peret, T. C., et al. 1998. J. Gen. Virol. 79:2221-2229) to resolve discrepant results. Specific sequences were detected by RT-PCR assays for the HRSV glycoprotein G gene in 26 (81.2%) of the 32 discordant samples including 11 A and 15 B strains (Table 18). Using the latter test as "gold standard", the sensitivity, specificity, positive predictive and negative predictive values of the multiplex real-time PCR assay and the antigenic test for HRSV were 94.5%, 98.9%, 99.0%, 94.0% and 81.6%, 94.7%, 94.7%, 81.8%, respectively. Four (66.7%) of the six false-negative HRSV results by multiplex PCR were positive for influenza A (Table 18). The mean C_T values of the PCR-positive/antigen-positive and PCR-positive/antigen-negative samples were 28.6 and 32.7, respectively (Table 18). These values correspond to approximately 1000 and 100 copies, respectively, when a specific HRSV plasmid was tested in the multiplex PCR assay.

Discussion

In this report, we describe a new multiplex real-time PCR assay for the most commonly detected respiratory viral pathogens, namely influenza viruses (A and B) and HRSV. The key features of this novel assay include its rapidity (turnaround time of approximately 2h30 including 30 min for sample preparation, 60 min for RT and 60 min for real-time PCR including viral identification), and its sensitivity (50 copies per assay for each viral target). Our results clearly demonstrate that our multiplex real-time PCR assay is more sensitive than commercially-available antigenic detection tests for both influenza A and HRSV with the greatest difference in sensitivity noted for influenza viruses.

Multiplex RT-PCR assays targeting up to nine different respiratory pathogens have been previously reported (Fan, J., K. J. Henrickson, and L. L. Savatski. 1998.. Clin. Infect. Dis. 26:1397-1402, Grondahl, B., et al. 1999. J. Clin. Microbiol. 37:1-7, Kehl, S. C., et al. 2001. 39:1696-1701, Osiowy, C. 1998. J. Clin. Microbiol. 36:3149-3154, Stockton, J., et al. . 1998. M J. Clin. Microbiol. 36:2990-2995, Valassina, M., A. M.

Cippone, M. G. Cusi, and P. E. Valensin. 1996. Clin. Diag. Virol. 8:227-232). More recently, a TaqMan-based real-time PCR assay for simultaneous detection of influenza A and B viruses has also been described (van Elden, L. J., et al. 2001. J. Clin. Microbiol. 39:196-200). Compared to conventional multiplex PCR assays, our real-time PCR test is much more rapid because it avoids additional nested amplification and/or hybridization steps required for identification of viral products. Furthermore, the real-time amplification procedure minimizes the chances of contamination because there is no post-PCR processing of the samples. Although our multiplex real-time PCR assay based on melting curve analysis of amplicons does not permit absolute quantification of the viral targets as in the TaqMan PCR procedure (van Elden, L. J., et al. 2001. J. Clin. Microbiol. 39:196-200), it allows for a larger number of viral targets to be simultaneously detected since there is no limitation related to the capability of the system to detect multiple dyes linked to detection probes with distinct emission wavelengths. Eventually, more than three viral targets could be simultaneously detected in the LightCycler assay assuming the absence of interaction between PCR primers and a reproducible and discriminative Tm value for each viral amplicon.

Evaluation of our multiplex real-time PCR assay in a pediatric population with severe ARTI clearly illustrated its clinical potential. Indeed, our assay identified a viral pathogen (influenza or HRSV) in two-thirds of the hospitalized children. These numbers are similar to the ones previously reported by our group in the same population using individual real-time assays for these viral pathogens (Boivin, G., et al. 2003. Emerg. Infect. Dis. 9:634-640). Notably, we were able to show that 6% of hospitalized children had dual viral infections, a finding that has been reported albeit at a lower frequency by other investigators (Grondahl, B., et al. 1999. J. Clin. Microbiol. 37:1-7, Kehl, S. C., et al. 2001. 39:1696-1701, Osiowy, C. 1998. J. Clin. Microbiol. 36:3149-3154). Rapid identification of theses viral pathogens (influenza and HRSV) is of paramount importance for isolation purposes in the hospital setting and early institution of specific antiviral therapy (Whitley, R. J., et al. 2001.. Pediatr. Infect. Dis. J. 20:127-133). We are currently designing additional multiplex assays

based on the same technology to cover the whole spectrum of respiratory pathogens including parainfluenza viruses (PIV), adenoviruses, enteroviruses, and coronaviruses (Treanor, J. 2002. Respiratory Infections, p. 7-26, In Richard. J. Whitley, Douglas D. Richman and Frederick G. Hayden (eds.). Clinical Virology. 2nd ed. ASM Press. Washington, D.C.). To this list, we should also add the recently described human metapneumovirus which has been reported by our group and others in up to 10% of ARTI in hospitalized children (Boivin, G., et al. 2003. Emerg. Infect. Dis. 9:634-640, Jartti, T., B. van den Hoogen, R. P. Garofalo, A. D. Osterhaus, and O. Ruuskanen. 2002.. Lancet. 360:1393-1394, Nissen, M. D., et al 2002. Med. J. Aust. 176:188, van den Hoogen, B. G., J. C. de Jong, J. Groen, T. Kuiken, R. de Groot, R. A. Fouchier, and A. D. Osterhaus. 2001.. Nat. Med. 7:719-724):

A specific evaluation of the real-time multiplex PCR assay for influenza A revealed that it was twice as sensitive as the rapid antigen detection test in use at our institution during the winter season of 2001-2002. None of the two PCR-negative, antigen-positive samples were confirmed by a second PCR targeting another conserved gene (M2) of influenza A whereas most (22/25, 88.0%) multiplex PCR-positive, antigen-negative were. In general, the false-negative antigenic test results could be explained by low amounts of viral RNA in those NPA samples as demonstrated by high C_T values in the corresponding real-time PCR assays. Three multiplex PCR test results were not confirmed by the influenza A M2 PCR test and were thus considered false-positive results. However, as we have not formally evaluated the sensitivity of the M2 PCR test and since viral cultures were not routinely done, we cannot rule out the possibility that such discrepant results were indeed true positive. We and other investigators have shown the superiority of RT-PCR tests over conventional methods (antigenic tests and viral culture) for detecting influenza viruses in clinical samples including those sent to the laboratory by mail (Boivin, G., I. Hardy, and A. Kress. 2001. J. Clin. Microbiol. 39:730-732, Ellis, J. S., D. M. Fleming, and M. C. Zambon. 1997. 1996. J. Clin. Microbiol. 35:2076-2082, Kehl, S. C., et al. 2001. 39:1696-1701, Steininger, C., et al. 2002. J. Clin. Microbiol. 40:2051-2056, van Elden, L. J., et al. 2001. J. Clin. Microbiol. 39:196-200). It is now common procedure at our institution

to confirm any negative antigenic tests results for influenza A and B by either viral culture or RT-PCR considering the high specificity but relatively poor sensitivity of the former assays.

For HRSV, the multiplex real-time PCR assay was also found to be more sensitive (by approximately 15%) than the rapid antigenic test when a second RT-PCR assay for the HRSV gG gene was used to resolve the discrepancies. Most of the false-negative antigenic test results could be explained by low amounts of viral RNA in the NPA samples as shown by mean C_T values of 32.7 for discordant results compared to 28.6 for concordant positive results. The specificity of the multiplex assay was excellent with only one positive result not confirmed by the second PCR test. In contrast, the multiplex PCR assay missed six cases of HRSV which tested positive in both the antigenic test and the second PCR assay for HRSV gG. Notably, four of those six samples tested positive for influenza A in the multiplex PCR assay. Thus, although our multiplex PCR test has the capability of detecting dual infections (as seen in 6% of our cases), it is possible that high amounts of one virus could inhibit amplification of other pathogens. It is noteworthy that the multiplex PCR assay had the ability to amplify both HRSV genotypes as shown by the detection of 15 B and 11 A genotypes using specific PCR assays aimed at detecting variable regions of the HRSV gG gene (Mazzulli, T., et al, 1999. J. Infect. Dis. 180:1686-1689). Although the rapid antigenic test had a relatively good performance (sensitivity: 82%, specificity 95%) for detection of HRSV in young children, the real advantage of PCR probably resides in the adult population where viral titers are lower and where antigenic tests are not recommended with upper respiratory tract samples (Englund, J. A., et al 1996.. 34:1649-1653).

There are some limitations to our study which are worth mentioning. First, the absence of circulation of influenza B viruses during the study period prevents adequate validation of our multiplex assay for this pathogen and in particular for its ability to detect mixed (influenza B and HRSV) infections. Second, the performance of our multiplex PCR assay cannot be generalized to other types of samples (throat

swabs, sputum) and to other populations (outpatients, adults) at the present time. Also, the absence of serological testing in our study may have underestimated the rate of viral infections especially for those children who presented late after the onset of symptoms. Finally, although it was relatively easy to discriminate between the different viral pathogens based on their specific Tm values only (Table 14), we sometimes had to confirm viral amplicons by gel electrophoresis, which increases the assay's turnaround time.

In conclusion, we described a rapid and sensitive multiplex real-time PCR assay for detecting influenza viruses and HRSV in children's NPA samples. This new assay is as specific and much more sensitive than currently-available antigen detection tests and could complement the latter in the hospital setting when there is high clinical suspicion despite negative results. Future work is needed to expand the panel of viral pathogens detected by such rapid molecular methods in order to eventually circumvent the need for viral cultures. Also, future evaluation of the multiplex PCR assay is warranted in the adult population with severe ARTI.

Example 10 : Description and Evaluation of the Multiplex Respiratory-1 PCR.

Same as example 9, except that hMPV primers (as defined in SEQ. ID. Nos. 95 and 96 or 112 and 114) were added to the multiplex PCR assay (already containing primers of SEQ. ID. Nos. 139+140 for influenza A, 141+142 for influenza B, and 143+144 for hRSV) at a concentration of 0.3 µM. The cycling conditions included an amplification step of 50 cycles of 15 s at 94°C, 5 s at 58°C, and 28 s at 72°C. The Tm values determined with the LightCycler instrument were 91°C, 85°C, 83°C, 79°C, and 82°C/77°C for the internal control, influenza A, influenza B, hRSV, and hMPV N/L genes, respectively (Fig. 7A for hMPV N primers of SEQ. ID. Nos. 95 and 96 and Fig. 7B for hMPV L primers of SEQ. ID Nos. 112 and 114). The lower limit of detection of the multiplex respiratory-1 PCR was 10, 50, 100, and 100 copies for influenza A, influenza B, hRSV, and hMPV, respectively, in the presence of 500 copies of an internal control (plasmid flanked by influenza B complementary primer sequences). The multiplex respiratory-1 assay was found to be specific with no amplification of adenoviruses, enteroviruses, rhinoviruses, parainfluenza viruses 1-3, coronaviruses, herpes simplex virus type 1 and 2, varicella-zoster virus, cytomegalovirus, HHV-6, HHV-8, and Epstein-Barr virus.

The multiplex respiratory-1 PCR was evaluated with NPA samples from 248 children ≤3 years with acute respiratory tract infections and 108 adults ≥50 years with exacerbation of chronic obstructive pulmonary disease or pneumonia requiring hospitalization during the 2002-03 winter/spring period in Québec City, Canada. In children, the detection rate for influenza A, influenza B, hRSV, and hMPV was 9/248 (3.6%), 4/248 (1.6%), 123/248 (49.6%), and 13/248 (5.2%), respectively. Five (2.0%) of the 248 children had a mixed viral infection. None of the NPA samples was found to contain PCR-inhibitory substances as shown by successful amplification of the internal control in all negative samples. In adults, the detection rate for influenza A, influenza B, hRSV, and hMPV was 3/108 (2.8%), 0/108 (0%), 13/108 (12.0%), and

2/108 (1.8%), respectively. One patient had a mixed viral infection (influenza A and hMPV). No samples contained PCR-inhibitory samples.

Example 11: Description and evaluation of the multiplex respiratory-2 PCR.

Same as example 9, except that the multiplex PCR assay is composed of the following PCR primers for detection of parainfluenza-1 (SEQ. ID: Nos. 147 and 148), parainfluenza-3 (SEQ. ID. Nos. 151 and 152), human adenoviruses (SEQ. ID. Nos. 153 and 155), and rhinoviruses/enteroviruses (SEQ. ID. Nos. 160 and 161). Primers are added in the PCR reaction mixture at a concentration of 0.2 μM (SEQ. ID. Nos. 147 and 151), 0.3 μM (SEQ. ID. Nos. 153 and 155), 0.4 μM (SEQ. ID. Nos. 148 and 152), and 0.5 μM (SEQ. ID. Nos. 160 and 161). The cycling conditions included an amplification step of 50 cycles of 15 s at 94°C, 5 s at 58°C, and 26 s at 72°C. The Tm values determined with the LightCycler instrument were 91.0°C, 80.0°C, 77.5°C, 89.0°C, 82.0°C, and 85.0°C for the internal control, parainfluenza-1, parainfluenza-3, adenoviruses, rhinoviruses, and enteroviruses, respectively (Fig. 8). The lower limit of detection of the multiplex respiratory-2 PCR was 50, 100, 10, 50, and 200 copies for parainfluenza-1, parainfluenza-3, adenoviruses, rhinoviruses, and enteroviruses, respectively, in the presence of 500 copies of an internal control (plasmid flanked by rhinovirus/enterovirus complementary primer sequences). The multiplex respiratory-2 assay was found to be specific with no amplification of influenza A, influenza B, hRSV, hMPV, coronavirus, herpes simplex virus type 1 and 2, varicella-zoster virus, cytomegalovirus, HHV-6, HHV-8, and Epstein-Barr virus.

Example 12 : Description of real-time PCR assays for the SARS-coronavirus.

Same as example 9, except that the PCR assay is composed uniquely of primers (two different possible sets) for the SARS-coronavirus as defined in SEQ. ID. Nos. 156-157 and 158-159. The concentration of the primers in the PCR reaction mixture is 0.5 μM. The amplification step included 50 cycles of 15 s at 94°C, 5 s at 58°C, and 20 s at 72°C. The Tm for both PCR products is 83°C (Fig. 12).

Table 1: List of hMPV sequences revealed in the present invention

SEQ ID NO.	Isolate No.	Target gene
2	hMPV-118	Nucleoprotein
3	hMPV-174	Nucleoprotein
4	hMPV-176	Nucleoprotein
5	hMPV-193	Nucleoprotein
6	C-77807	Nucleoprotein
7	C-77748	Nucleoprotein
8	hMPV-217	Nucleoprotein
10	hMPV-163	Nucleoprotein
11	hMPV-174	Nucleoprotein
12	hMPV-184	Nucleoprotein
13	hMPV-185	Nucleoprotein
14	C-76837	Nucleoprotein
15	C-77807	Nucleoprotein
16	C-78123	Nucleoprotein
17	C-78132	Nucleoprotein
18	C-78163	Nucleoprotein
19	C-91205	Nucleoprotein
20	C-91364	Nucleoprotein
21	C-91830	Nucleoprotein
22	hMPV-Q86	Nucleoprotein
23	hMPV-Q97	Nucleoprotein
24	Can 97-83	Nucleoprotein
25	Can 98-75	Nucleoprotein
26	Can 98-75	Fusion type II
27	C-67345	Fusion type I
28	Can 97-83	Fusion type I
29	C-68661	Fusion type I
30	C-73750	Fusion type I
31	C-74549	Fusion type I
32	C-74783	Fusion type I
33	C-76837	Fusion type I
34	C-77748	Fusion type I
35	C-77807	Fusion type I
36	C-78132	Fusion type I
37	C-78163	Fusion type I
38	C-78406	Fusion type I
39	hMPV-174	Fusion type II
40	hMPV-193	Fusion type I
41	hMPV-194	Fusion type I
42	hMPV-198	Fusion type I
43	hMPV-217	Fusion type II
44	hMPV-208	Fusion type I
45	hMPV-223	Fusion type I
46	hMPV-228	Fusion type I

47	C-85885	Fusion type I
48	C-60298	Fusion type I
49	C-63778	Fusion type II
50	C-64199	Fusion type II
51	C-64200	Fusion type II
52	C-64536	Fusion type II
53	C-73769	Fusion type II
54	C-74501	Fusion type II
55	C-78429	Fusion type II
56	C-78562	Fusion type II
57	hMPV-118	Fusion type II
58	hMPV-200	Fusion type I
59	hMPV-176	Fusion type I
60	hMPV-193	Glycoprotein
61	hMPV-228	Glycoprotein
62	C-77748	Glycoprotein
63	C-78132	Glycoprotein
64	C-78406	Glycoprotein
65	C-73750	Glycoprotein
66	C-60298	Glycoprotein
67	C-74783	Glycoprotein
68	C-74549	Glycoprotein
69	C-77807	Glycoprotein
70	C-78163	Glycoprotein
71	C-68661	Glycoprotein
72	C-88448	Glycoprotein
73	C-88470	Glycoprotein
74	C-86316	Glycoprotein
76	hMPV-118	Polymerase
77	hMPV-163	Polymerase
78	hMPV-174	Polymerase
79	hMPV-184	Polymerase
80	hMPV-196	Polymerase
81	C-76837	Polymerase
82	C-77807	Polymerase
83	hMPV-185	Polymerase
84	C-78123	Polymerase
85	C-91205	Polymerase
86	C-91364	Polymerase
87	C-91749	Polymerase
88	hMPV-Q86	Polymerase
89	hMPV-Q97	Polymerase
90	Can 97-83	Polymerase

Table 2: List of hMPV PCR primers developed in the present invention

SEQ ID NO.	Target gene	Originating DNA Position[a]	Originating DNA SEQ ID NO.
91	Nucleoprotein (N)	25	1
92	Nucleoprotein (N)	97	1
93	Nucleoprotein (N)	146[b]	1
94	Nucleoprotein (N)	1025[b]	1
95	Nucleoprotein (N)	58	9
96	Nucleoprotein (N)	986[b]	9
97	Fusion (F)	3052	1
98	Fusion (F)	3810[b]	1
99	Fusion (F)	4671[b]	1
100	Matrix (M)	2165	1
101	Matrix (M)	2943[b]	1
102	Phosphoprotein (P)	1248	1
103	Phosphoprotein (P)	2124[b]	1
104	Glycoprotein (G)	6231	1
105	Glycoprotein (G)	6247	1
106	Glycoprotein (G)	6957[b]	1
107	Glycoprotein (G)	6981[b]	1
108	Glycoprotein (G)	6235	1
109	Glycoprotein (G)	7227[b]	1
110	RNA polymerase (L)	142	75
111	RNA polymerase (L)	691[b]	75
112	RNA polymerase (L)	7308	1
113	RNA polymerase (L)	7857[b]	1
114	RNA polymerase (L)	7616	1

[a] Position refers to nucleotide position of 5' end of primer. Oligonucleotides are 17-20 bp in length.

[b] Primer is reverse-complement of target sequence.

Table 3: List of hMPV probes developed in the present invention

SEQ ID NO.	Target gene	Originating DNA	
		Position[a]	SEQ ID NO.
115	Nucleoprotein (N)	786	1
116	Nucleoprotein (N)	808	1
117	Fusion (F)	3163	1
118	Fusion (F)	3187	1
119	Matrix (M)	2636	1
120	Matrix (M)	2659	1
121	Phosphoprotein (P)	1492	1
122	Phosphoprotein (P)	1518	1
123	Glycoprotein (G)	6509	1
124	Glycoprotein (G)	6530	1
125	RNA polymerase (L)	7600	1
126	RNA polymerase (L)	7627	1
127	RNA polymerase (L)	7331	1
128	Nucleoprotein (N)	824	9
129	RNA polymerase (L)	434	75

[a] Position refers to nucleotide position of 5'end of probe. Oligonucleotides are 21-25 bp in length.

Table 4: Length of amplicons obtained with different hMPV primer pairs which are objects of the present invention

SEQ ID NO.	Target Gene	Length of amplicon
91 + 94	Nucleoprotein (N)	1001 bp
92 + 94	Nucleoprotein (N)	929 bp
91 + 93	Nucleoprotein (N)	122 bp
97 + 99	Fusion (F)	1620 bp
97 + 98	Fusion (F)	759 bp
100 + 101	Matrix (M)	779 bp
102 + 103	Phosphoprotein (P)	877 bp
104 + 107	Glycoprotein (G)	751 bp
105 + 106	Glycoprotein (G)	711 bp
108 + 109	Glycoprotein (G)	993 bp
112 + 113	RNA polymerase (L)	550 bp
112+114	RNA polymerase (L)	308 bp

Table 5: Comparative evaluation of different hMPV primers for detection of hMPV strains

Strains No.	Type of sample	Year	N Primers SEQ. ID. Nos. 92+94		M primers SEQ. ID. Nos. 100+101		F primers SEQ. ID. Nos. 97+98		P primers SEQ. ID. Nos. 102+103		L primers SEQ. ID. Nos. 112 + 113	
	I or S		+/-	Tm (°C)	+/-	Tm (°C)	+/-	Tm (°C)	+/-	Tm (°C)	+/-	Tm (°C)
C-74501	I	2000	+	82.92	-		+	83.65	-		+	78.14
C-74783	I	2000	+	82.76	+	83.49	-		+	81.51	+	78.20
C-76837	I	2000	+	82.94	+	83.43	+	83.17	+	80.86	-	
C-77719	I	2001	+	83.26	+	84.64	-		-		+	77.87
C-77738	I	2001	+	82.99	-		-		-		+	77.51
C-77748	I	2001	+	83.06	+	82.96	+	83.10	+	81.27	+	77.38
C-77807	I	2001	+	82.99	+	83.26	+	83.25	+	80.65	+	78.34
C-78024	I	2001	+	82.86	+	83.95	-		-		-	
C-78028	I	2001	+	82.98	+	83.13	-		-		+	78.32
C-78123	I	2001	+	82.83	-		-		-		+	78.48
C-85984	I	2002	+	82.59	+	83.21	+	83.51	+	79.99	+	77.97
C-85448	I	2002	+	83.66	+	82.72	-		+	80.31	+	78.88
C-85983	I	2002	+	82.59	+	83.03	+	83.88	+	80.46	+	78.01
C-85988	I	2002	+	82.17	-		+	84.39	-		+	78.06
C-86316	I	2002	+	82.64	+	82.75	+	83.18	+	80.18	+	78.02
C-85126	I	2002	+	81.89	+	82.05	+	82.39	+	80.00	+	78.04
C-85473	I	2002	+	82.39	+	82.57	+	82.91	+	80.74	+	77.82
C-85332	I	2002	+	81.98	-		-		-		+	77.72
C-85596	I	2002	+	82.24	+	82.73	+	83.17	+	80.35	+	77.58
C-85542	I	2002	+	82.22	+	82.72	-		+	80.08	+	77.67
HMPV-118	S	2002	+	82.47	-		-		-		ND	ND
HMPV-174	S	2002	+	82.18	+	82.06	+	83.16	+	80.17	+	78.20
HMPV-176	S	2002	+	81.41	-		+	83.97	-		+	77.81
HMPV-193	S	2002	+	82.22	+	81.89	+	83.00	+	80.58	+	77.94
HMPV-194	S	2002	+	82.24	+	81.32	+	82.98	-		+	77.84
HMPV-198	S	2002	+	82.66	-		-		-		-	
HMPV-200	S	2002	+	82.53	-		+	83.46	-		+	77.62
HMPV-208	S	2002	+	82.11	+	81.81	+	83.33	+	80.06	+	78.40
HMPV-217	S	2002	+	82.13	+	81.58	+	83.01	-		+	77.76

HMPV-223	S	2002	+	81.89	+	82.36	+	83.04	-	+	78.10
Mean			82.63 ± 0.87		82.75 ± 0.80		83.29 ± 0.45		80.48 ± 0.46	77.99 ± 0.33	

Note: I = viral isolates; S = clinical specimens; Tm = melting temperature as determined by a LightCycler instrument.

Table 6: Size of the viral fusion (F) gene fragments obtained for different hMPV isolates following enzymatic digestion with restriction endonucleases

SEQ ID NO.	Fragments obtained after digestion with the restriction endonuclease		F genotype[a]
	ApaL I	Avr II	
35	532 bp + 227 bp	759 bp	I
58	532 bp + 227 bp	759 bp	I
39	759 bp	447 bp + 312 bp	II
40	532 bp + 227 bp	759 bp	I
41	532 bp + 227 bp	759 bp	I
43	759 bp	447 bp + 312 bp	II
44	532 bp + 227 bp	759 bp	I
59	532 bp + 227 bp	759 bp	I
45	532 bp + 227 bp	759 bp	I

[a]As determined by DNA sequencing and phylogenetic studies.

Table 7. Type of laboratory confirmation by type of infection

Laboratory test	hMPV		hRSV		Influenza A		Adenovirus		PIV 2	
	Tests done	Positive	Tests Done	Positive	Tests done	Positive	Tests done	Positive	Tests done	Positive
PCR	208	12 5.8%	208	106 51.0%	208	45 21.6%	NA	NA	NA	NA
Culture	100	2 2%	100	31 31%	100	14 14%	100	6 6%	100	1 1%
Antigen detection (Immunofluorescence)	NA	NA	NA	NA	NA	NA	81	1 1%	76	1 1.3%
Antigen detection (ELISA)	NA	NA	204	94 46.1%	100	14 14%	NA	NA	NA	NA
Total (+) in at least one test	12 (5.8%)		118 (56.7%)		49 (23.6%)		6 (6%)		2 (2%)	

Delay between onset of symptoms and NPA, days					
Mean/median	6.3/5.0	5.2/4.0	8.7/5.0	6.0/6.5	3.0/3.0

Note: hMPV, human metapneumovirus; hRSV, human respiratory syncytial virus; PIV, parainfluenza virus; NPA, nasopharyngeal aspirate; NA, not available.

Table 8. Signs and symptoms by type of viral infection

Signs and symptoms	HMPV	hRSV	Influenza A	Single virus	Multiple viruses	No virus detected	Total
	N=12	N=118	N=49	N=141	N=23	N=44	N=208
Fever	8	67	38	85	17	25	127
	67%	57%	78%	60%	74%	57%	61%
Cough	12	117	47	138	23	40	201
	100%	99%	96%	98%	100%	90%	97%
Rhinorrhea	11	107	41	123	22	42	187
	92%	91%	84%	87%	96%	96%	90%
Retractions	11	112	40	125	22	39	186
	92%	95%	82%	89%	96%	89%	89%
Wheezing	10	77	28	83	19	31	133
	83%	65%	57%	59%	83%	71%	64%
Lacrymation	3	37	15	46	6	11	63
	25%	31%	31%	33%	26%	25%	30%
Diarrhea	1	19	13	24	5	10	39

	8%	17%	27%	17%	22%	23%	19%
Vomiting	3	9	5	10	4	1	15
	25%	8%	10%	7%	17%	2%	7%
Other	0	31	9	33	4	9	46
		26%	18%	23%	17%	21%	22%

Note: hMPV, human metapneumovirus; hRSV, human respiratory syncytial virus.

Table 9. Final diagnoses (complications) by type of viral infection

Complications	hMPV	HRSV	Influenza A	Single virus	Multiple viruses	No virus detected	Total
	N=12	N=118	N=49	N=141	N=23	N=44	N=208
Bronchiolitis	8	99	25	98	19	25	142
	66.7%	83.9%	51.0%	69.5%	82.6%	56.8%	68.3%
Otitis	6	70	27	78	15	24	117
	50.0%	59.3%	55.1%	55.3%	65.2%	54.6%	56.3%
Pneumonia	2	30	18	39	7	12	58
	16.7%	25.4%	36.7%	27.7%	30.4%	27.3%	27.9%
Laryngotracheobronchitis	0	10	6	9	4	1	14
		8.5%	12.2%	6.4%	17.4%	2.3%	6.7%
Sinusitis	0	3	3	2	2	1	5
		2.5%	6.1%	1.4%	8.7%	2.3%	2.4%
Pharyngitis	0	1	0	2	0	2	4
		0.9%		1.4%		4.6%	1.9%
Flu syndrome	0	2	0	2	0	4	6
		1.7%		1.4%		9.1%	2.9%

Croup	0	2	0	2	0	1	3
		1.7%		1.4%		2.3%	1.4%
Other	1	4	3	9	0	5	14
	8.3%	3.4%	6.1%	6.4%		11.4%	6.7%

Note: hMPV, human metapneumovirus; hRSV, human respiratory syncytial virus.

Table 10: List of PCR primers used for amplification of respiratory viruses

Primer SEQ ID NO.	Virus	Target Gene	Originating DNA Position[a]	Originating DNA SEQ ID NO.
139	Influenza A	Matrix	32 / 7	130-131
140	Influenza A	Matrix	276[b] / 251[b]	130-131
141	Influenza B	Matrix	82	132
142	Influenza B	Matrix	605[b]	132
143	RSV	Fusion	1111	133
144	RSV	Fusion	1490[b]	133
95	hMPV	Nucleocapsid	58	9
96	hMPV	Nucleocapsid	986[b]	9
110	hMPV	Polymerase	142	75
111	hMPV	Polymerase	691[b]	75
145	PIV-1	Nucleocapsid	64	134
146	PIV-1	Nucleocapsid	147[b]	134
149	PIV-3	Nucleocapsid	416	135
150	PIV-3	Nucleocapsid	649[b]	135
154	Adenovirus	Hexon	154	136
155	Adenovirus	Hexon	368[b]	136
160	Entero/Rhinovirus	5'-non coding region	94 / 71	137/138
161	Entero/Rhinovirus	5'-non coding region / VP2	746[b] / 603[b]	137/138
141	IC[c] multiplex 1	DNA polymerase HSV-2	1	170
142	IC[c] multiplex 1	DNA polymerase HSV-2	558[b]	170
160	IC[c] multiplex 2	DNA polymerase HSV-2	1	171
161	IC[c] multiplex 2	DNA polymerase HSV-2	554[b]	171
112	hMPV	Polymerase	7308	1
114	hMPV	Polymerase	7616	1
147	PIV-1	Nucleocapsid	822	134
148	PIV-1	Nucleocapsid	1029	134
151	PIV-3	Nucleocapsid	792	135
152	PIV-3	Nucleocapsid	902	135
153	Adenovirus	Hexon	7	136
156	Sars-CoV	nsp9		
157	Sars-CoV	nsp9		
158	Sars-CoV	nsp11		
159	Sars-CoV	nsp11		

[a] Position refers to nucleotide position of 5'-end of primer. Oligonucleotides are 16-26 bp in length.

[b] Primer is reverse-complement of target sequence.

[c] IC refers to the internal control used to monitor the efficiency of the PCR amplification

Table 11: List of viral probes developed in the present invention

Probe SEQ ID NO.	Target	Originating DNA Position[a]	Originating DNA SEQ ID NO.
162	Influenza A	114[b] / 89[b]	130/131
163	Influenza B	383	132
164	hRSV	1391[b]	133
128	hMPV	824	9
129	hMPV	434	75
165	PIV-1	96	134
166	PIV-3	616[b]	135
167	Adenovirus	228	136
168	Rhinovirus	467[b]	138
169	Enterovius	644[b]	137
172	Internal Control[c]	321[b] / 325[b]	170/171

[a]Position refers to nucleotide position of 5'-end of primer. Oligonucleotides are 20-23 bp in length.

[b]Probe is reverse-complement of target sequence.

[c]A modified DNA polymerase gene sequence of herpes simplex virus (HSV) type 2 serves as the internal control template.

Table 12: Length of amplicons obtained with the different viral primer pairs

Primer SEQ ID NO.	Virus	Target gene	Length of amplicons
139 + 140	Influenza A	Matrix	245 bp
141 + 142	Influenza B	Matrix	524 bp
143 + 144	hRSV	Fusion	380 bp
95 + 96	hMPV	Nucleocapsid	929 bp
110 + 111	hMPV	Polymerase	550 bp
145 + 146	PIV-1	Nucleocapsid	84 bp
149 + 150	PIV-3	Nucleocapsid	234 bp
154 + 155	Adenoviruses	Hexon	215 bp
160 + 161	Rhinoviruses	5'-non coding region – VP2	533 bp
160 + 161	Enteroviruses	5'-non coding region	653 bp
141 + 142	IC multiplex 1	DNA polymerase HSV-2	558 bp
160 + 161	IC multiplex 2	DNA polymerase HSV-2	554 bp
112 + 114	hMPV	Polymerase	308 bp
147 + 148	PIV-1	Nucleocapsid	208 bp
151 + 152	PIV-3	Nucleocapsid	111 bp
153 + 155	Adenovirus	Hexon	362 bp
156 + 157	Sars-CoV	nsp9	198 bp
158 + 159	Sars-CoV	nsp11	368 bp

Table 13: Selection of primer sets with resulting melting temperature (Tm) values of the corresponding amplicons for each multiplex assay

Primer SEQ ID NO.	Target	Multiplex NO.	Tm (°C)
139 + 140	Influenza A	1	85
141 + 142	Influenza B	1	83
143 + 144	hRSV	1	79
95 + 96 or 112 + 114	hMPV	1	82 or 77
141 + 142	Internal Control	1	91
147 + 148	Parainfluenza 1	2	80.0
151 + 152	Parainfluenza 3	2	77.5
153 + 155	Adenovirus	2	89.0
160 + 161	Rhinovirus	2	82.0
160 + 161	Enterovirus	2	85.0
160 + 161	Internal Control	2	91

Table 14. Evaluation of the multiplex PCR respiratory assay with temporally-different strains of influenza (Flu) and human respiratory syncytial (HRSV) viruses.

Virus	Subtype / Genotype[a]	Year	Type	Strain	Tm (°C)
Flu A	H1	99-00	clinical	-	85.10
Flu A	H1	99-00	clinical	-	85.39
Flu A	H1	00-01	clinical	-	85.12
Flu A	H1	00-01	clinical	-	85.20
Flu A	H1	00-01	clinical	-	85.04
Flu A	H1	01-02	clinical	-	84.62
Flu A	H1	01-02	clinical	-	85.07
Flu A	H1	02-03	clinical	-	85.50
Flu A	H1	02-03	clinical	-	85.29
Flu A	H1	95	vaccine	A/Beijing/262/95	85.06
Flu A	H1	33	vaccine	A/WSN/33	85.25
Mean AH1					**85.15±0.22**
Flu A	H3	98-99	clinical	-	85.58
Flu A	H3	98-99	clinical	-	85.18
Flu A	H3	98-99	clinical	-	85.35
Flu A	H3	98-99	clinical	-	85.57
Flu A	H3	98-99	clinical	-	85.37
Flu A	H3	01-02	clinical	-	85.07
Flu A	H3	01-02	clinical	-	85.65
Flu A	H3	02-03	clinical	-	84.92
Flu A	H3	99	vaccine	A/Panama/2007/99	85.18
Flu A	H3	97	vaccine	A/Sydney/5/97	85.06
Mean AH3					**85.31±0.25**
Mean FluA					**85.27±0.22**
Flu B	-	98-99	clinical	-	83.87
Flu B	-	98-99	clinical	-	83.12
Flu B	-	00-01	clinical	-	83.01
Flu B	-	00-01	clinical	-	83.03
Flu B	-	00-01	clinical	-	82.88
Flu B	-	00-01	clinical	-	83.75
Flu B	-	00-01	clinical	-	83.17
Flu B	-	02-03	clinical	-	83.89
Flu B	-	02-03	clinical	-	83.84
Flu B	-	97	vaccine	B/Harbin/7/97	84.12
Mean FluB					**83.47±0.46**

Table 14. Evaluation of the multiplex PCR respiratory assay with temporally-different strains of influenza (Flu) and human respiratory syncytial (HRSV) viruses. (Continued).

HRSV	B	99-00	clinical	-	79.32
HRSV	A	99-00	clinical	-	79.83
HRSV	A	00-01	clinical	-	79.06
HRSV	A	00-01	clinical	-	78.85
HRSV	B	01-02	clinical	-	79.34
HRSV	B	01-02	clinical	-	79.52
HRSV	B	01-02	clinical	-	79.98
HRSV	B	01-02	clinical	-	79.52
HRSV	A	01-02	clinical	-	79.84
HRSV	B	01-02	clinical	-	79.42
HRSV	B	01-02	clinical	-	79.49
HRSV	B	01-02	clinical	-	79.48
HRSV	B	01-02	clinical	-	79.55
HRSV	B	01-02	clinical	-	79.32
HRSV	A	01-02	clinical	-	79.77
HRSV	A	01-02	clinical	-	79.92
HRSV	A	01-02	clinical	-	79.40
Mean HRSV-A					**79.52±0.43**
Mean HRSV-B					**79.49±0.19**
Mean HRSV					**79.51±0.30**

[a] Subtyping was performed by multiplex PCR for the influenza hemagglutinin gene (2) whereas genotyping was done by conventional PCR for the HRSV G gene (22)

Table 15. Evaluation of the multiplex PCR respiratory assay for detection of influenza A (Flu A) virus.

		Antigenic test (Directigen Flu A+B) (n = 172)		Flu A M2 PCR assay [a] (n = 27)	
		+	-	+	-
Multiplex PCR	+	17	25	22	3
	-	2	128	0	2

Sensitivity: 39/39 = 100%

Specificity: 130/133 = 97.7%

Positive predictive value: 39/42 = 92.8%

Negative predictive value: 130/130 = 100%

[a] Only performed for samples showing discordant results with the multiplex PCR assay and the antigenic test.

Table 16: Evaluation of discordant test results for influenza A (Flu A) virus.

Sample No.	Multiplex PCR Flu A results (C_T)[a]	Antigenic test Flu A results	M2 PCR Flu A results	Other PCR-amplified viruses[b]
16	-	+	-	HRSV
23	+(27)	-	+	
24	-	+	-	HRSV
27	+(28)	-	+	HRSV
35	+(28)	-	+	HRSV
36	+(32)	-	+	
40	+(34)	-	+	
43	+(36)	-	+	HRSV
44	+(35)	-	+	
50	+(34)	-	+	
51	+(33)	-	+	HRSV
103	+(35)	-	+	
107	+(35)	-	+	
114	+(37)	-	-	
118	+(38)	-	+	HRSV
120	+(36)	-	+	HRSV
125	+(38)	-	+	
130	+(35)	-	+	HRSV
131	+(36)	-	-	
132	+(24)	-	+	HRSV
134	+(36)	-	-	
141	+(28)	-	+	
152	+(34)	-	+	
158	+(32)	-	+	
160	+(33)	-	+	HRSV
161	+(28)	-	+	HRSV
163	+(32)	-	+	

[a] Cycle threshold value as determined by LightCycler.

[b] Viruses amplified by the multiplex PCR assay i. e. influenza (Flu) A and B as well as the human respiratory syncytial virus (HRSV).

Table 17: Evaluation of the multiplex PCR respiratory assay for detection of human respiratory syncytial virus (HRSV).

		Antigenic test (RSV TestPack) (n = 204)		HRSV gG PCR assays[a] (n = 32)	
		+	-	+	-
Multiplex PCR	+	83	21	20	1
	-	11	89	6	5

Sensitivity: 103/109 = 94.5%
Specificity: 94/95 = 98.9%
Positive predictive value: 103/104 = 99.0%
Negative predictive value: 94/100 = 94.0%

[a] Only performed for samples showing discordant results with the multiplex PCR assay and the antigenic test.

Table 18: Evaluation of discordant test results for human respiratory syncytial virus (HRSV).

Sample No.	Multiplex PCR HRSV results (C_T)[a]	Antigenic test HRSV results	Glycoprotein G PCR HRSV results (genotype)	Other PCR-amplified viruses[b]
16	+(32)	-	+(B)	
17	+(27)	-	+(B)	
26	+(36)	-	+(B)	
34	-	+	-	
35	+(33)	-	+(B)	Flu A
43	+(36)	-	+(A)	Flu A
46	+(34)	-	+(B)	
55	+(34)	-	+(B)	
58	+(33)	-	+(B)	
62	-	+	+(A)	Flu A
70	+(37)	-	+(B)	
71	+(35)	-	+(B)	
72	+(34)	-	+(B)	
73	+(26)	-	+(A)	
79	+(33)	-	+(B)	
80	-	+	-	
91	+(35)	-	+(A)	
95	+(29)	-	+(B)	
100	+(30)	-	+(A)	
112	-	+	-	
129	+(34)	-	+(B)	
130	+(35)	-	+(B)	Flu A
134	-	+	+(A)	Flu A
139	-	+	+(A)	
142	-	+	+(A)	Flu A
144	-	+	+(B)	Flu A
150	+(34)	-	-	
187	+(31)	-	+(A)	
191	+(29)	-	+(A)	

Table 18: Evaluation of discordant test results for human respiratory syncytial virus (HRSV). (Continued).

193	-	+	+ (A)
211	-	+	-
212	-	+	-

[a] Cycle threshold value as determined by Lightcycler.

[b] Viruses amplified by the multiplex PCR assay i. e. influenza (Flu) A and B as well as the human respiratory syncytial virus (HRSV).

CLAIMS:

1- A method for detecting and/or quantifying human metapneumovirus (hMPV) in a sample said method comprising:

-providing at least one probe or primer specific for a nucleic acid sequence of said hMPV;

-contacting said at least one probe and/or primer with said sample to allow annealing of said probe and/or primer with said nucleic acid sequence; and

-detecting and/or quantifying said nucleic acid sequence using said annealed probe and/or primer.

2- The method as claimed in claim 1 wherein said nucleic acid sequence is comprised in a hMPV gene selected from a group consisting of nucleocapsid (N), phosphoprotein (P), matrix (M), fusion (F), membrane (M2), polymerase (L), glycoprotein (G) and small hydrophobic (SH) genes.

3- The method as claimed in claim 2 wherein said nucleic acid sequence is selected from any one of SEQ ID NO 2-8, 10-74, 76-90 or fragments thereof.

4- The method as claimed in claim 3 wherein said probe or primer is at least 10 nucleotides in length.

5- A method for detecting and/or quantifying human metapneumovirus (hMPV) in a sample said method comprising:

-performing reverse transcription using a template RNA derived from said sample to obtain a nucleic acid product;

-amplifying said nucleic acid product using a pair of oligonucleotide primers wherein said primers are specific for a sequence comprised in a hMPV gene selected from a group consisting of nucleocapsid (N), phosphoprotein (P), matrix (M), fusion

(F), membrane (M2), polymerase (L), glycoprotein (G) and small hydrophobic (SH) genes to produce an amplification product; and

-detecting and/or quantifying said amplification product.

6- The method as claimed in claim 5 wherein said oligonucleotide primers are at least 10 nucleotides in length and capable of hybridizing with any one of SEQ ID 2-8, 10-74, 76-90.

7- The method as claimed in claim 5 or 6 wherein said step of performing reverse transcription comprises contacting said template RNA with a primer selected from SEQ ID Nos 91-114 and wherein said oligonucleotide primers for PCR are selected from SEQ ID Nos 91-114.

8- The method as claimed in any one of claim 5-7 wherein said oligonucleotide primers are pairs of primers selected from SEQ ID Nos 91 and 94, 92 and 94, 91 and 93, 97 and 99, 97 and 98, 100 and 101, 102 and 103, 104 and 107, 105 and 106, 108 and 109, 112 and 113 and 112 and 114.

9- The method as claimed in claim 5 wherein said template RNA is contacted with primer selected from SEQ ID NO 91-94 and 112-114 and wherein said oligonucleotide primers are selected from SEQ ID NO 91-94 and 112-114.

10- The method as claimed in any one of claim 5-9 wherein said step of amplifying is performed using PCR.

11- The method as claimed in claim 10 wherein said PCR is RT-PCR.

12- The method as claimed in claim 11 wherein said RT-PCR is real time RT-PCR.

13- The method as claimed in claim 11 or 12 wherein said RT-PCR comprises detecting said amplification products by characteristic melting temperature of said amplification products.

14- The method as claimed in claim 11 or 12 wherein said RT-PCR comprises detecting and/or quantifying said amplification products with probes specific for said products.

15- The method as claimed in claim 14 wherein said probes are selected from SEQ ID no 115-129.

16- A composition for detecting a hMPV said composition comprising a combination of oligonucleotides for performing reverse transcription of a template RNA from said hMPV virus and for an amplification reaction of a product obtained from said reverse transcription wherein said oligonucleotides for said amplification reaction are specific for a sequence comprised in a hMPV gene selected from a group consisting of nucleocapsid (N), phosphoprotein (P), matrix (M), fusion (F), membrane (M2), polymerase (L), glycoprotein (G) and small hydrophobic (SH) genes.

17- The composition of claim 16 wherein said oligonucleotides for said amplification reaction are primers selected from the group comprising SEQ ID Nos 91-114 and combination thereof.

18- A kit comprising a combination of oligonucleotides wherein the oligonucleotides are selected from:

SEQ ID NOs: 91-114 and the pairs of SEQ ID Nos 91 and 94, 92 and 94, 91 and 93, 97 and 99, 97 and 98, 100 and 101, 102 and 103, 104 and 107, 105 and 106, 108 and 109, 112 and 113 and 112 and 114.

19- A method for typing an hMPV strain which comprises the steps of:

-amplifying an hMPV nucleic acid fragment and detecting specific viral sequences;

-digesting said fragment with one or more restriction endonuclease; and

-analysing the restriction fragment length polymorphisms.

20- The method as claimed in claim 19 wherein said nucleotide sequences are found in fusion(F) or glycoprotein (G) gene.

21- The method as claimed in claim 20 wherein said sequences are selected from SEQ ID NO 26-74.

22- A method for detecting and/or quantifying two or more respiratory viruses in a sample said method comprising:

-providing at least two probes and/or primers specific for a nucleic acid sequences in said two or more respiratory viruses;

-contacting said at least two probes and/or primers with said sample to allow annealing of said probes and/or primers with said nucleic acid sequences; and

-detecting and/or quantifying said nucleic acid sequences using said annealed probes and/or primers.

23- The method as claimed in claim 22 wherein at least one of said nucleic acid sequences is comprised in a hMPV gene selected from a group consisting of nucleocapsid (N), phosphoprotein (P), matrix (M), fusion (F), membrane (M2), polymerase (L), glycoprotein (G) and small hydrophobic (SH) genes.

24- The method as claimed in claim 23 wherein said nucleic acid sequence is selected from any one of SEQ ID NO 2-8, 10-74, 76-90 or fragments thereof.

25- The method as claimed in claim 24 wherein said probes and/or primers are at least 10 nucleotides in length.

26- A method for detecting and/or quantifying two or more respiratory viruses in a sample said method comprising:

-performing reverse transcription using a template RNA derived from said sample to obtain nucleic acid products for each of said viruses;

-amplifying said nucleic acid products using two or more pairs of oligonucleotide primers to produce an amplification product; and

-detecting and/or quantifying said amplification products.

27- The method as claimed in claim 26 wherein at least one of said primers is specific for a sequence comprised in a hMPV gene selected from a group consisting of nucleocapsid (N), phosphoprotein (P), matrix (M), fusion (F), membrane (M2), polymerase (L), glycoprotein (G) and small hydrophobic (SH) genes.

28- The method as claimed in claim 27 wherein said oligonucleotide primers specific for a sequence comprised in a hMPV gene are capable of hybridizing with any one of SEQ ID 2-8, 10-74, 76-90.

29- The method as claimed in claim 28 wherein said oligonucleotide primers are at least 10 nucleotides in length.

30- The method as claimed in claim 29 wherein said oligonucleotide primer specific for a sequence comprised in a hMPV gene are selected from SEQ ID Nos 91-114.

31- The method as claimed in any one of claim 26-30 wherein said at least two respiratory viruses comprise influenza A and B, human respiratory syncytial viruses A and B, parainfluenza viruses (PIV) types 1-4, adenoviruses, rhinoviruses,

enteroviruses and coronaviruses or any combination thereof and wherein said primers and said two or more viruses are selected such that the detection and or quantification is specific for each of said viruses.

32- The method as claimed in claim 31 wherein said oligonucleotide primers are at least 10 nucleotides in length and capable of hybridizing with any one of SEQ ID NO 130-138 and 170-171.

33- . The method as claimed in claim 31 or 32 wherein said at least two viruses are influenza A, human Respiratory syncytial virus, human metapneumovirus and influenza B.

34- The method as claimed in claim 31 or 32 wherein said at least two viruses are Parainfluenza 1, Rhinovirus, Adenovirus, Parainfluenza 3, Enterovirus, and coronavirus.

35- The method as claimed in claim 31 wherein said pairs of oligonucleotide primers are selected from SEQ ID Nos 139 and 140, 141 and 142, 143 and 144, 92 and 94, 112 and 113, 112 and 114, 145 and 146, 147 and 148, 149 and 150, 151 and 152, 154 and 155, 153 and 155, 156 and 157, 158 and 159,160 and 161.

36- The method as claimed in any one of claim 26-35 wherein said step of amplifying is performed using PCR and said primer are selected such that the products of the amplifications can be distinguished.

37- The method as claimed in claim 36 wherein said PCR is RT-PCR.

38- The method as claimed in claim 37 wherein said RT-PCR is real time RT-PCR.

39- The method as claimed in claim 37 or 38 wherein said RT-PCR comprises detecting said amplification products by characteristic melting temperature of said amplification products.

40- The method as claimed in claim 37 or 38 wherein said RT-PCR comprises detecting and/or quantifying said amplification products with probes specific for said products.

41- The method as claimed in claim 40 wherein said probes are selected from SEQ ID no 162-169 and 172.

42- A composition for detecting at least two respiratory viruses said viruses comprising at least hMPV said composition comprising a combination of oligonucleotides for performing reverse transcription of a template RNA from said at least two respiratory viruses and for an amplification reaction of a product obtained from said reverse transcription wherein said oligonucleotides for said amplification reaction comprise oligonucleotides specific for a sequence comprised in a hMPV gene selected from a group consisting of nucleocapsid (N), phosphoprotein (P), matrix (M), fusion (F), membrane (M2), polymerase (L), glycoprotein (G) and small hydrophobic (SH) genes.

43- The composition of claim 42 wherein said oligonucleotides for said amplification reaction are primers selected from the group comprising SEQ ID Nos 91-114 and combination thereof.

44- A method for simultaneously detecting two or more respiratory tract viruses in a sample, said method comprising:

Selecting primers specific for a nucleic acid sequence in each of said two or more viruses, said primers to be used in an amplification reaction for detection of said nucleic acid sequences;

distributing said primers in one or more assay mixture comprising said sample and at least two pairs of primers; and

wherein said selecting and said distributing is based on said detection of said nucleic acid sequence of said two or more viruses being substantially free of overlap and enabling to distinguish between said two or more viruses.

45- The method as claimed in claim 44 wherein said amplification reaction is PCR.

46- The method as claimed in claim 45 wherein said PCR is RT-PCR.

47- The method as claimed in claim 45 wherein said RT-PCR is real time RT-PCR.

48- The method as claimed in claim 24 wherein said detection is melting temperature.

49- An isolated nucleic acid comprising a sequence that hybridizes under stringent conditions to a hybridization probe the nucleotide sequence of which consists of any one of SEQ ID NO 2-8, 10-74, 76-90 or complements thereof.

50- A kit comprising a nucleic acid probe that is at least 10 nucleotides in length and is selected from a group consisting sequences hybridizable to SEQ ID Nos 2-8, 10-74, 76-90 or fragments thereof.

51- A human metapneumovirus (hMPV) deposited with the International Depository Authority of Canada under Accession number IDAC 111203-01 (CAN97-83).

52- A human metapneumovirus (hMPV) deposited with the International Depository Authority of Canada under Accession number IDAC 111203-02 (CAN98-75).

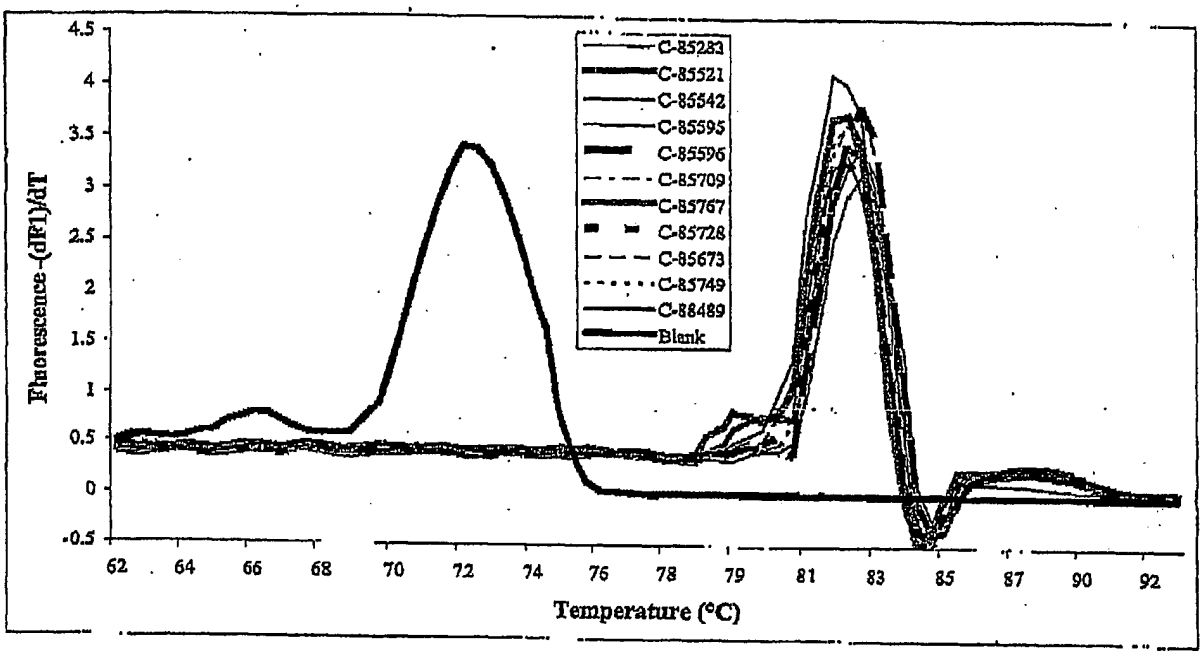

Fluorescence -(dF1)/dT
Temperature (°C)
C-85283
C-85521
C-85542
C-85595
C-85596
C-85709
C-85767
C-85728
C-85673
C-85749
C-88489
Blank
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
-0.5
62
64
66
68
70
72
74
76
78
79
81
83
85
87
90
92

FIGURE 1

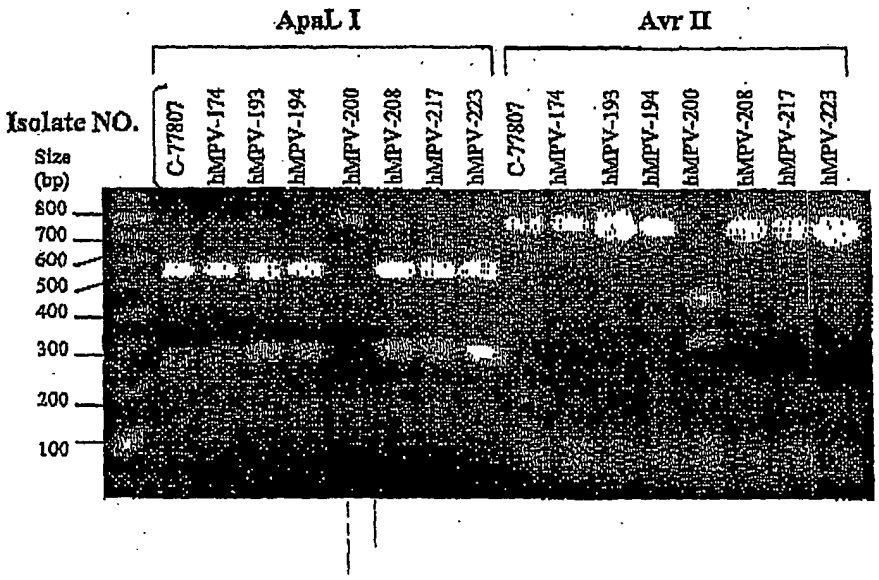

ApaL I
Avr II
Isolate NO.
Size (bp)
C-77807
hMPV-174
hMPV-193
hMPV-194
hMPV-200
hMPV-208
hMPV-217
hMPV-223
C-77807
hMPV-174
hMPV-193
hMPV-194
hMPV-200
hMPV-208
hMPV-217
hMPV-223
800
700
600
500
400
300
200
100

FIGURE 2

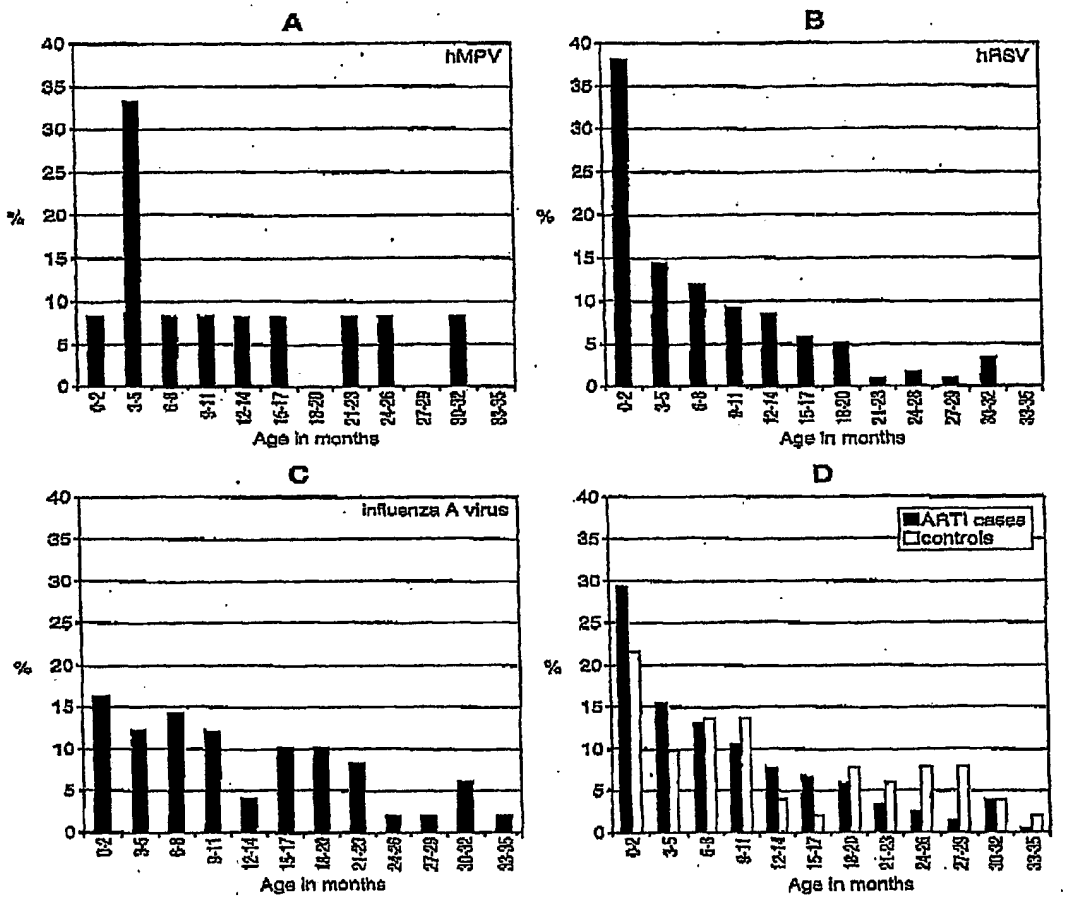

A
hMPV
%
Age in months
B
hRSV
%
Age in months
C
influenza A virus
%
Age in months
D
ARTI cases
controls
%
Age in months
0-2
3-5
6-8
9-11
12-14
15-17
18-20
21-23
24-26
27-29
30-32
33-35

FIGURE 3

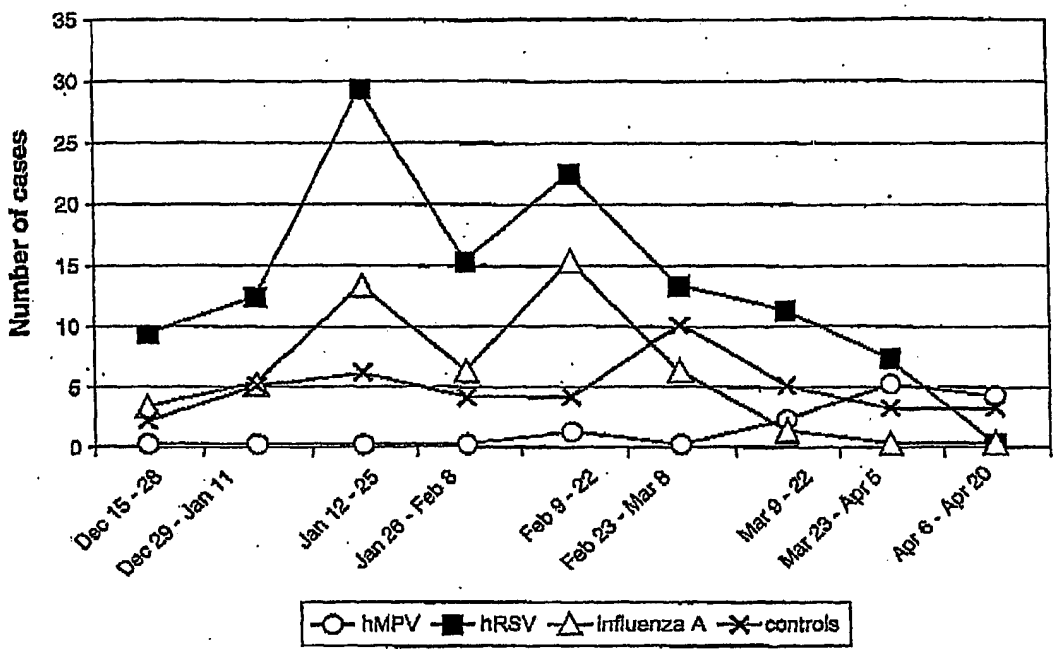

35
30
25
20
15
10
5
0
Number of cases
Dec 15 - 28
Dec 29 - Jan 11
Jan 12 - 25
Jan 26 - Feb 8
Feb 9 - 22
Feb 23 - Mar 8
Mar 9 - 22
Mar 23 - Apr 5
Apr 6 - Apr 20
hMPV
hRSV
Influenza A
controls

FIGURE 4

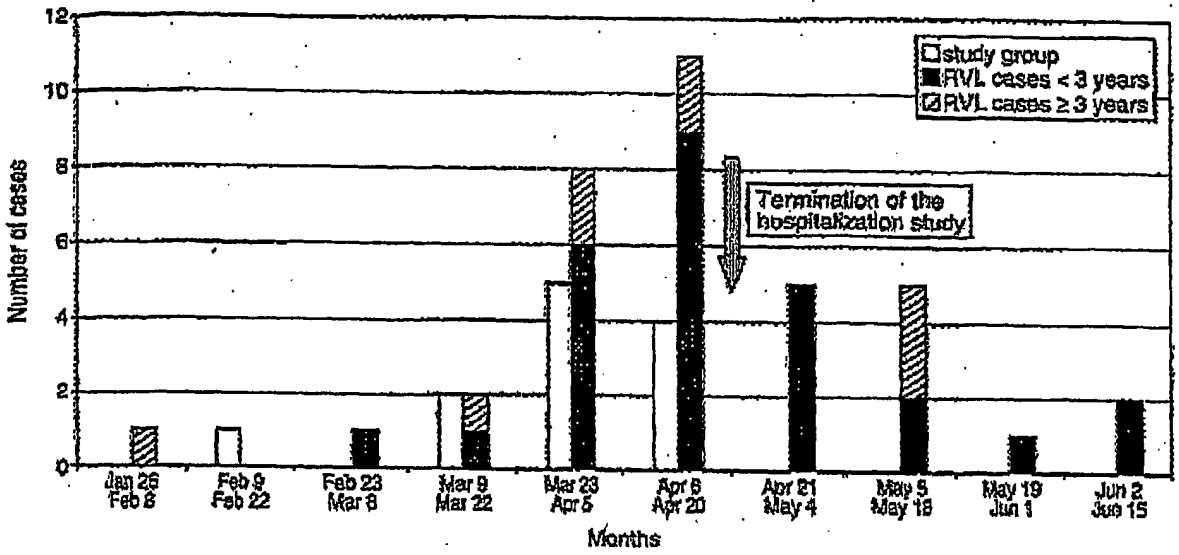

12
10
8
6
4
2
0
Number of cases
study group
RVL cases < 3 years
RVL cases ≥ 3 years
Termination of the hospitalization study
Jan 26 Feb 8
Feb 9 Feb 22
Feb 23 Mar 8
Mar 9 Mar 22
Mar 23 Apr 5
Apr 6 Apr 20
Apr 21 May 4
May 5 May 18
May 19 Jun 1
Jun 2 Jun 15
Months

FIGURE 5

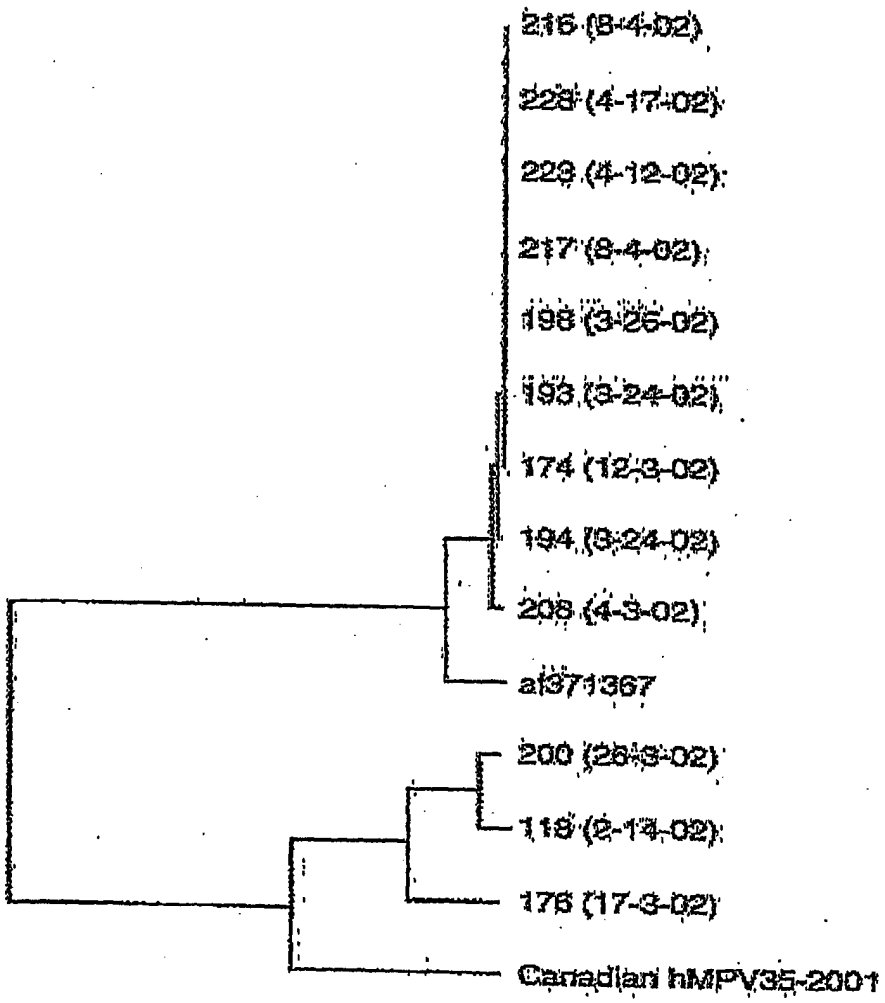
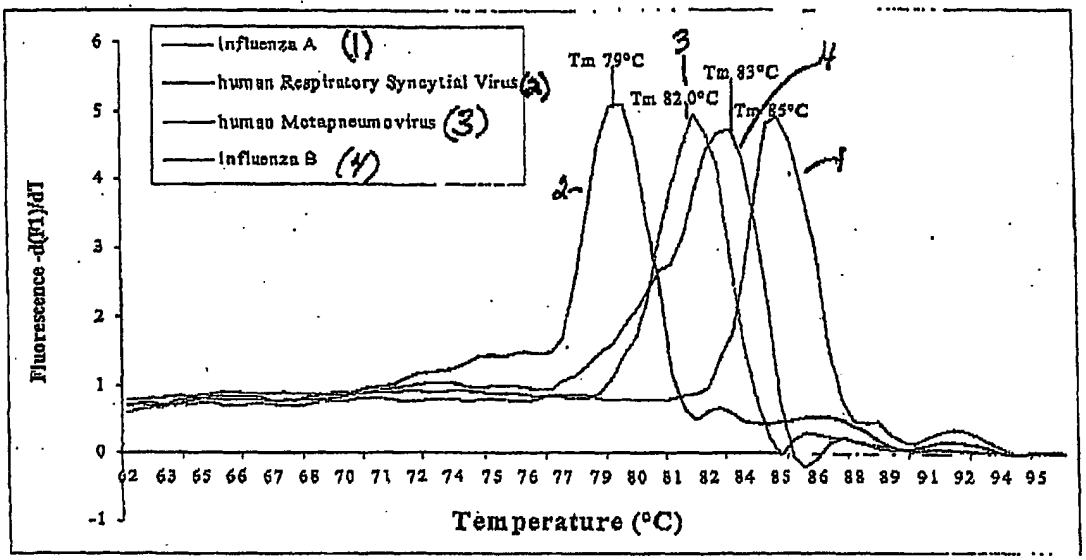

216 (8-4-02)
228 (4-17-02)
229 (4-12-02)
217 (8-4-02)
198 (3-26-02)
193 (3-24-02)
174 (12-3-02)
194 (3-24-02)
208 (4-3-02)
a1371367
200 (26-3-02)
118 (2-14-02)
176 (17-3-02)
Canadian hMPV35-2001

FIGURE 6

A


Influenza A (1)
human Respiratory Syncytial Virus (2)
human Metapneumovirus (3)
Influenza B (4)
Tm 79°C
Tm 82.0°C
Tm 83°C
Tm 85°C
Fluorescence -d(F1)/dT
Temperature (°C)

B

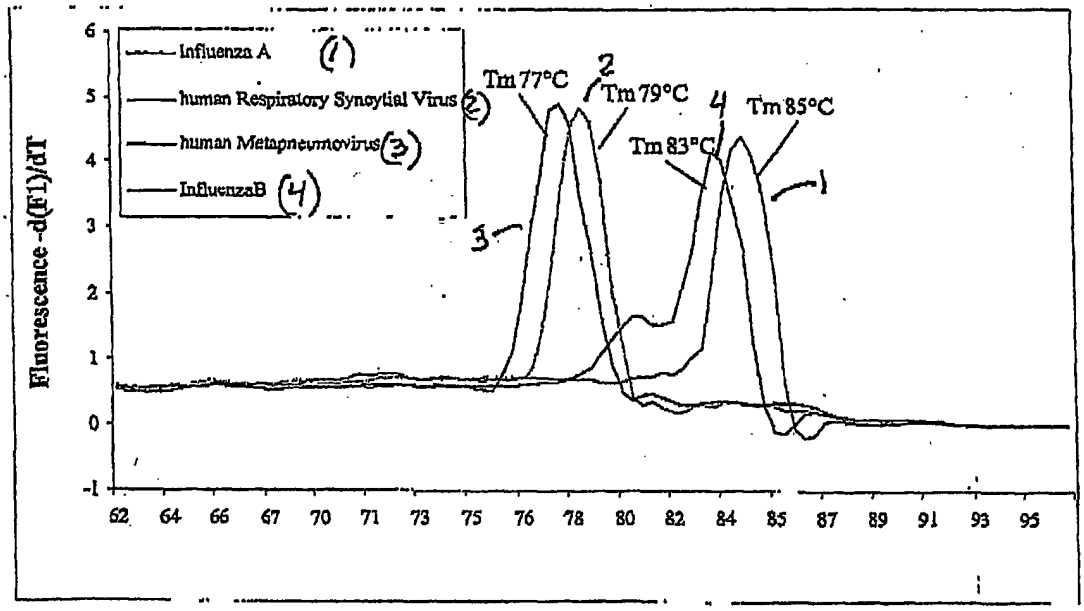

Influenza A (1)
human Respiratory Syncytial Virus (2)
human Metapneumovirus (3)
InfluenzaB (4)
Tm 77°C
Tm 79°C
Tm 83°C
Tm 85°C
Fluorescence -d(F1)/dT

FIGURE 7

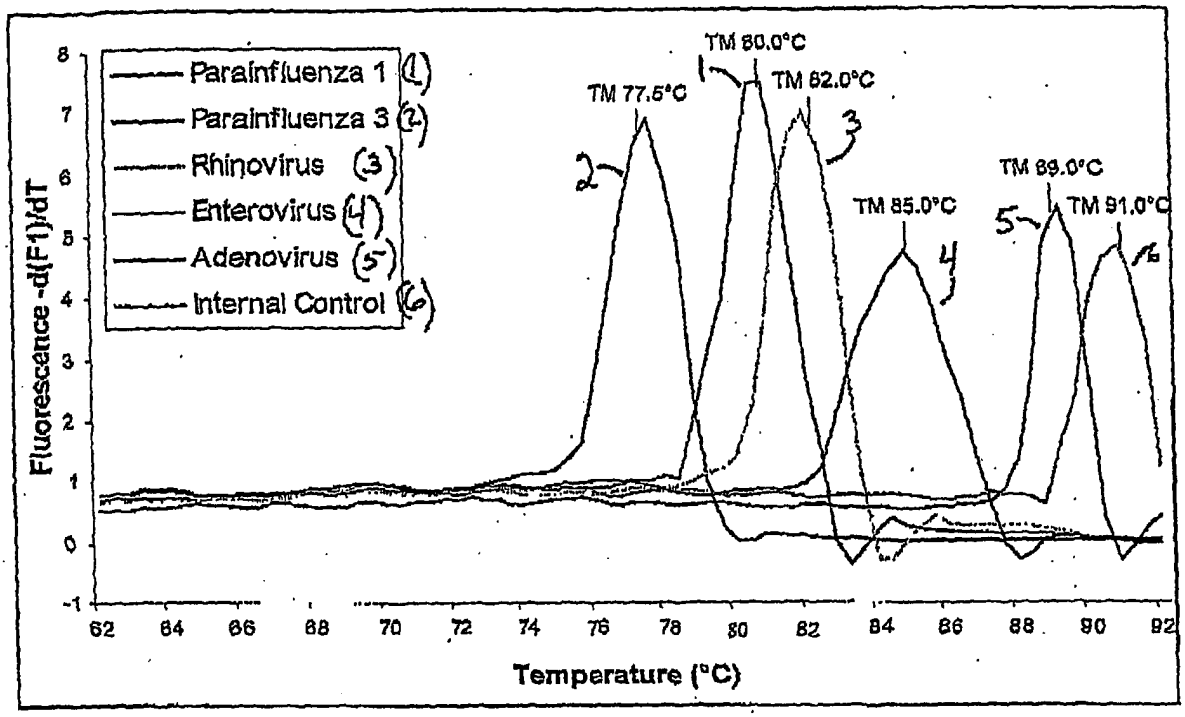

Parainfluenza 1 (1)
Parainfluenza 3 (2)
Rhinovirus (3)
Enterovirus (4)
Adenovirus (5)
Internal Control (6)
TM 77.5°C
TM 80.0°C
TM 82.0°C
TM 85.0°C
TM 89.0°C
TM 91.0°C
Fluorescence -d(F1)/dT
Temperature (°C)

FIGURE 8

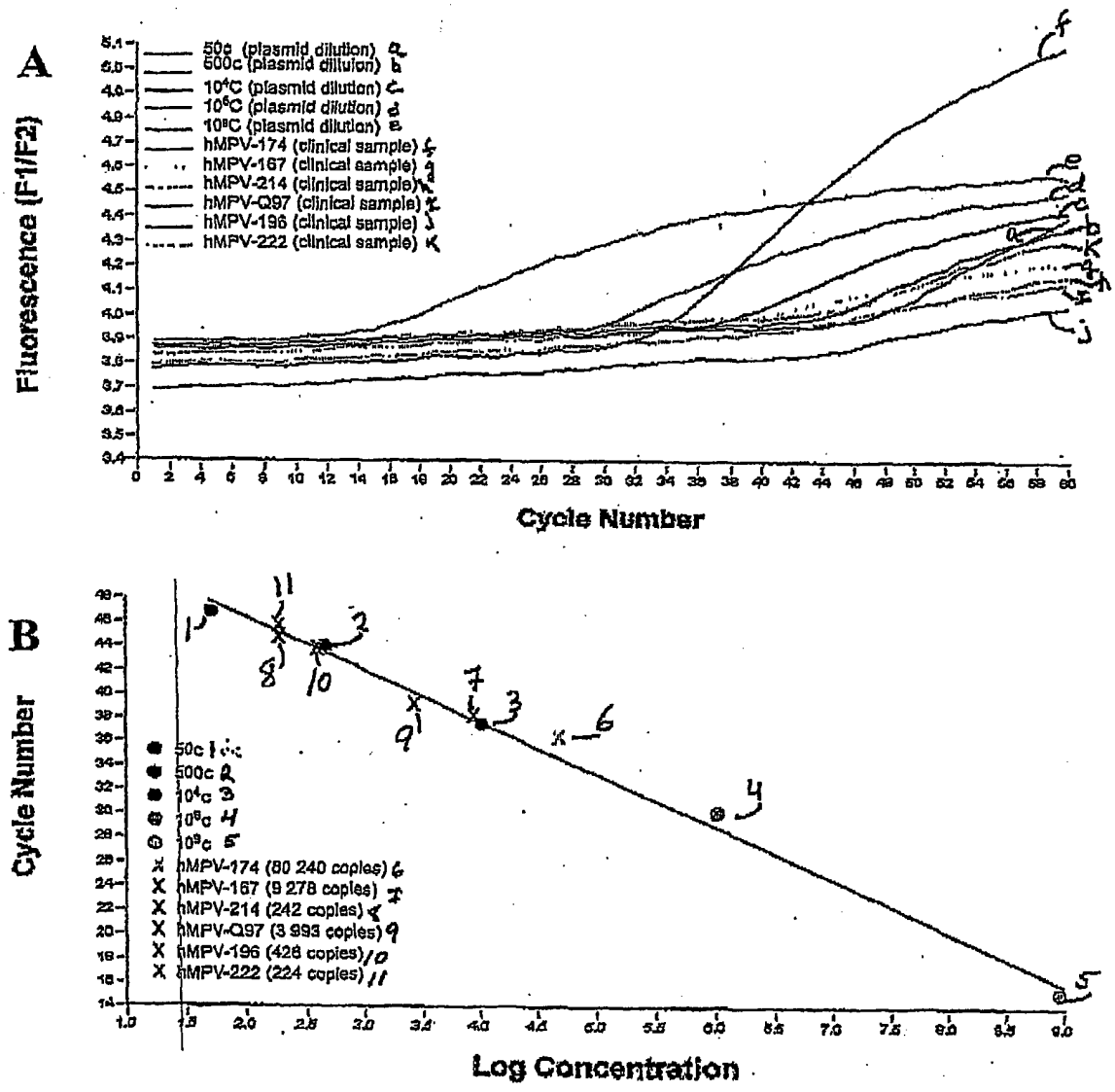

A
Fluorescence (F1/F2)
50c (plasmid dilution)
500c (plasmid dilution)
10⁴C (plasmid dilution)
10⁵C (plasmid dilution)
10⁸C (plasmid dilution)
hMPV-174 (clinical sample)
hMPV-167 (clinical sample)
hMPV-214 (clinical sample)
hMPV-Q97 (clinical sample)
hMPV-196 (clinical sample)
hMPV-222 (clinical sample)
Cycle Number
B
Cycle Number
50c
500c
10⁴c
10⁵c
10⁸c
hMPV-174 (80 240 copies)
hMPV-167 (9 278 copies)
hMPV-214 (242 copies)
hMPV-Q97 (3 993 copies)
hMPV-196 (426 copies)
hMPV-222 (224 copies)
Log Concentration

FIGURE 9

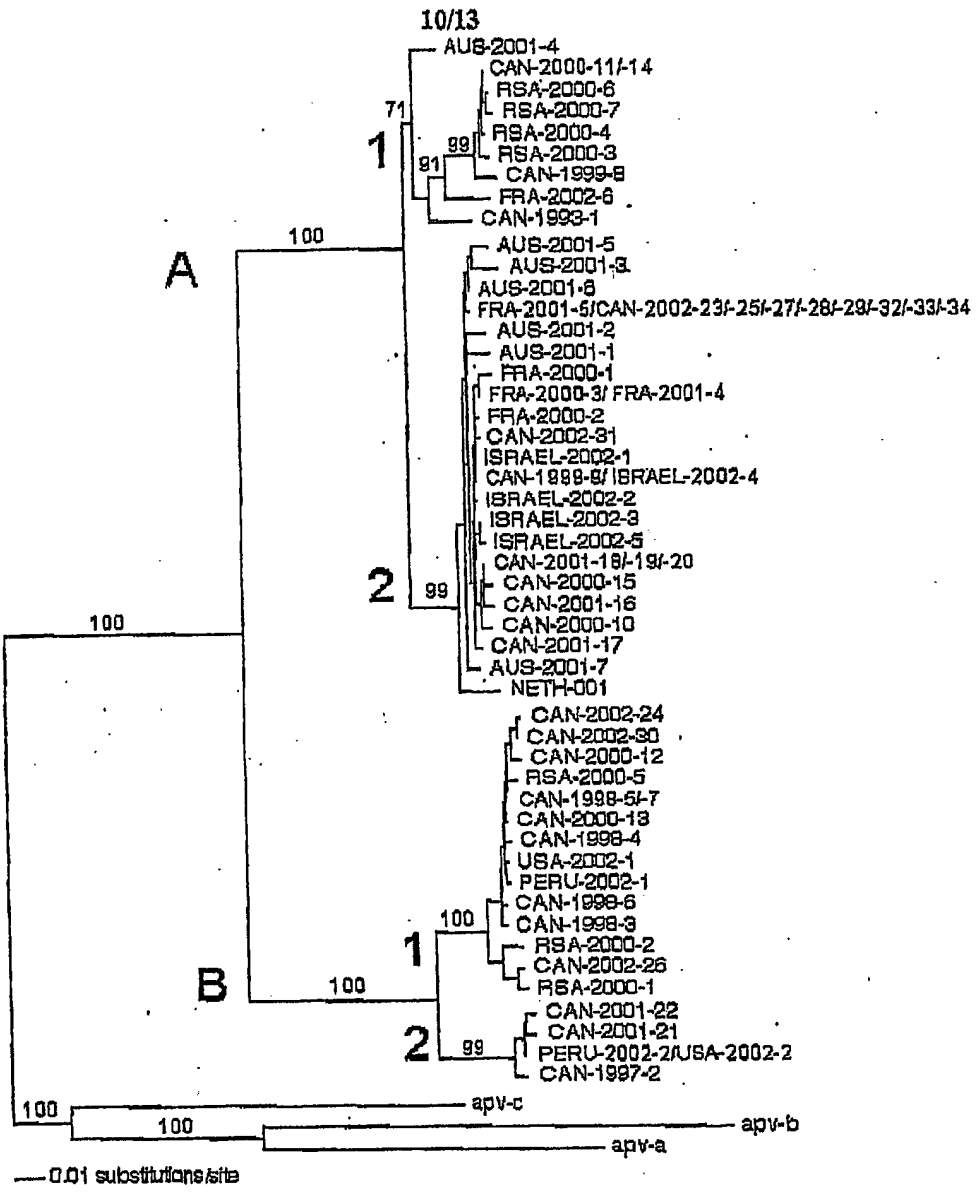

AUS-2001-4
CAN-2000-11/-14
RSA-2000-6
RSA-2000-7
RSA-2000-4
RSA-2000-3
CAN-1999-8
FRA-2002-6
CAN-1993-1
AUS-2001-5
AUS-2001-3
AUS-2001-6
FRA-2001-5/CAN-2002-23/-25/-27/-28/-29/-32/-33/-34
AUS-2001-2
AUS-2001-1
FRA-2000-1
FRA-2000-3/ FRA-2001-4
FRA-2000-2
CAN-2002-31
ISRAEL-2002-1
CAN-1998-9/ ISRAEL-2002-4
ISRAEL-2002-2
ISRAEL-2002-3
ISRAEL-2002-5
CAN-2001-18/-19/-20
CAN-2000-15
CAN-2001-16
CAN-2000-10
CAN-2001-17
AUS-2001-7
NETH-001
CAN-2002-24
CAN-2002-30
CAN-2000-12
RSA-2000-5
CAN-1998-5/-7
CAN-2000-13
CAN-1998-4
USA-2002-1
PERU-2002-1
CAN-1998-6
CAN-1998-3
RSA-2000-2
CAN-2002-26
RSA-2000-1
CAN-2001-22
CAN-2001-21
PERU-2002-2/USA-2002-2
CAN-1997-2
apv-c
apv-b
apv-a
0.01 substitutions/site
A
B
1
2
1
2
71
99
91
100
99
100
100
100
100
100
99

FIGURE 10 A

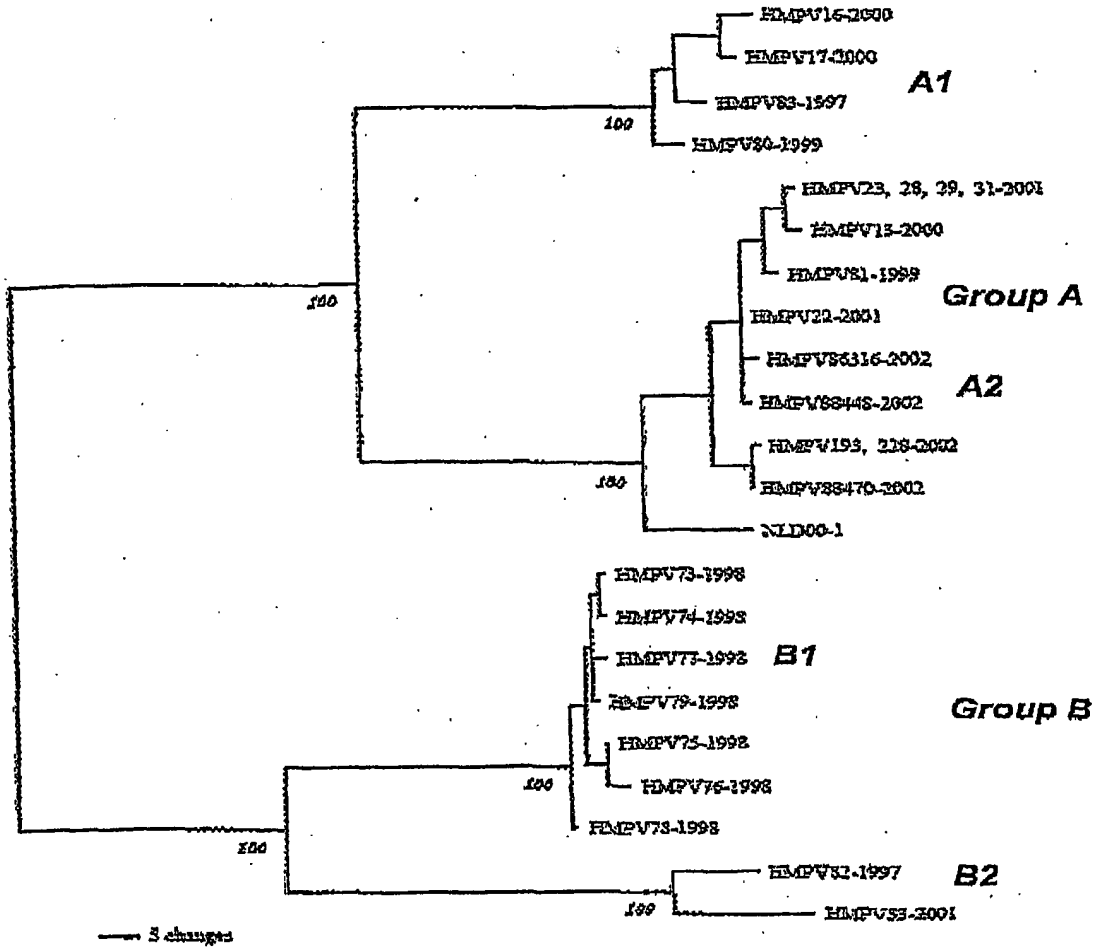

HMPV16-2000
HMPV17-2000
HMPV83-1997
HMPV80-1999
A1
100
HMPV23, 28, 29, 31-2001
HMPV15-2000
HMPV81-1999
Group A
HMPV22-2001
HMPV86316-2002
HMPV88448-2002
A2
HMPV193, 228-2002
HMPV88470-2002
NLD00-1
100
100
HMPV73-1998
HMPV74-1998
HMPV77-1998
B1
HMPV79-1998
HMPV75-1998
HMPV76-1998
HMPV78-1998
Group B
100
HMPV82-1997
HMPV83-2001
B2
100
5 changes

FIGURE 10 B

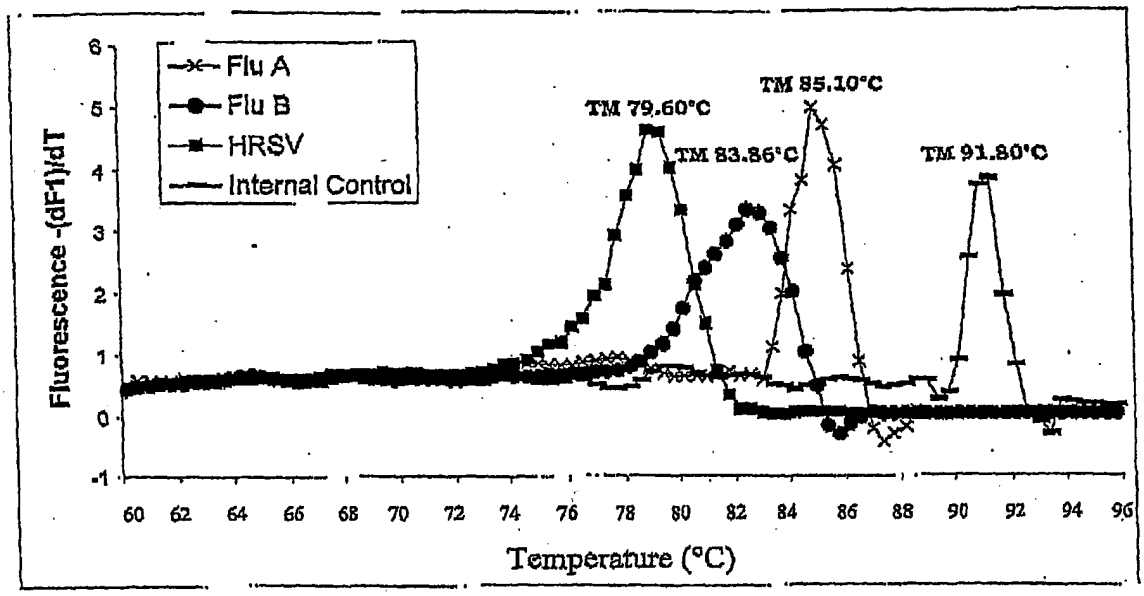

Flu A
Flu B
HRSV
Internal Control
TM 79.60°C
TM 83.86°C
TM 85.10°C
TM 91.80°C
Fluorescence -(dF1)/dT
Temperature (°C)
6
5
4
3
2
1
0
-1
60
62
64
66
68
70
72
74
76
78
80
82
84
86
88
90
92
94
96

FIGURE 11

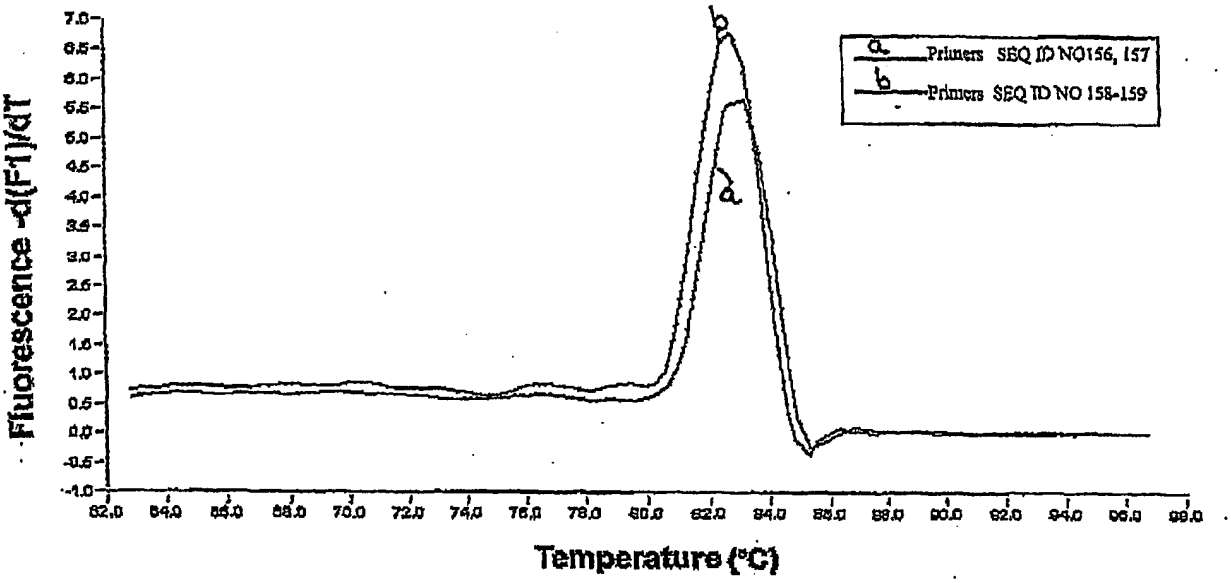

Fluorescence -d(F1)/dT
Temperature (°C)
a Primers SEQ ID NO156, 157
b Primers SEQ ID NO 158-159

FIGURE 12

SEQUENCE LISTING

<110> Boivin, Guy

<120> METHOD FOR DETECTING RESPIRATORY VIRUSES

<130> 6013-148PCT

<150> CA 2,411,264
<151> 2002-12-19

<150> CA 2,418,004
<151> 2003-01-24

<160> 172

<170> FastSEQ for Windows Version 4.0

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<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(13450)
<223> human metapneumovirus, strain 00-1
Accession number af371337

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caatatatta gcacagcttt aggatcagag agagtgcagc agattctgag gaactcaggc 300
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aaccgtgtac taagtgatgc actcaagaga tacctagaa tggacatacc aaagattgcc 660
agatccttct atgacttatt tgaacaaaaa gtgtatcaca gaagtttgtt cattgagtat 720
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ttcatgcaag cttatggggc cggtcaaaca atgctaaggt ggggggtcat tgccaggtca 840
tccaacaata taatgttagg acatgtatcc gtccaagctg agttaaaaca ggtcacagaa 900
gtctatgact tggtgcgaga aatgggccct gaatctggac ttctacattt aaggcaaagc 960
ccaaaagctg gactgttatc actagccaac tgtcccaact ttgcaagtgt tgtttctcgga 1020
aatgcctcag gcttaggcat aatcggtatg tatcgaggga gagtaccaaa cacagaatta 1080
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<211> 878
<212> DNA
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<220>
<221> gene

<222> (1)...(878)
<223> human metapneumovirus, strain Quebec Isolate No hMPV-174

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<211> 876
<212> DNA
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<220>
<221> gene
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<223> human metapneumovirus, strain Quebec Isolate No hMPV-176

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<220>
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<222> (1)...(869)
<223> human metapneumovirus, strain Qubec Isolate No hMPV-193

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<223> human metapneumovirus, strain Quebec Isolate No C-77807

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<211> 850
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(850)
<223> human metapneumovirus, strain Quebec Isolate No hMPV-217

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gtgaagtcca agtggtctta accagaacgt actctctggg gaaagttaaa aacaataaag 240
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acaaagaagc aaggaaaaca atggcaacct tgcttaagga atcatcaggt aatatcccac 360
aaaatcagag gccctcagca ccagacacac ccataatctt attatgtgta ggtgccttaa 420
tatttactaa gctagcatca accatagaag tgggactaga gaccacagtc agaagggcta 480
accgtgtact aagtgatgca ctcaagagat accctagaat ggacatccca aaaattgcca 540
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gtaaagcatt aggatcatca tctacaggca gcaaagcaga aagtctattt gttaatatat 660
tcatgcaagc ttatggagcc ggtcaaacaa tgctaaggtg gggggtcatt gccaagtcat 720
ccaacaatat aatgttagga catgtatccg tccaagctga attaaaaaca ggtcccagaa 780
gtccatgact tggtgcgaga aatggggcct gaatctggac ttctactttt aaggcaaagc 840
ccaaaagctg 850

<210> 9
<211> 1185
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(1185)
<223> human metapneumovirus, strain 00-1, accession number af371337

<400> 9
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caacaagaaa taacactgtt gtgtggagaa attctgtatg ctaaacatgc tgactacaaa 180
tatgctgcag aaataggaat acaatatatt agcacagctt taggatcaga gagagtgcag 240
cagattctga ggaactcagg cagtgaagtc caagtggtct taaccagaac gtactctctg 300
gggaaaatta aaaacaataa aggagaagat ttacagatgt tagacataca cggggtagag 360
aagagctggg tagaagagat agacaaagaa gcaaggaaaa caatggcaac cttgcttaag 420
gaatcatcag gtaatatccc acaaaatcag aggccctcag caccagacac acccataatc 480
ttattatgtg taggtgcctt aatattcact aaactagcat caaccataga agtgggacta 540
gagaccacag tcagaagggc taaccgtgta ctaagtgatg cactcaagag atacccтaga 600
atggacatac caaagattgc cagatccttc tatgacttat ttgaacaaaa agtgtatcac 660
agaagtttgt tcattgagta tggcaaagca ttaggctcat catctacagg cagcaaagca 720
gaaagtctat ttgttaatat attcatgcaa gcttatgggg ccggtcaaac aatgctaagg 780
tgggggtca ttgccaggtc atccaacaat ataatgttag gacatgtatc cgtccaagct 840
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<210> 10
<211> 700
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(700)
<223> human metapneumovirus, strain Quebec Isolate No hMPV-163

<400> 10
tgcagaaata ggaatacaat atattagcac agctttagga tcagagagag tgcagcagat 60
tctgaggaac tcaggcagtg aagtccaagt ggtcttaacc agaacgtact ctctggggaa 120
agttaaaaac aataaaggag aagatttaca gatgttagac atacacgggg tagagaagag 180
ctgggtagaa gagatagaca aagaagcaag gaaaacaatg gcaaccttgc ttaaggaatc 240
atcaggtaat atcccacaaa atcagaggcc ctcagcacca gacacaccca taatcttatt 300
atgtgtaggt gccttaatat ttactaaact agcatcaacc atagaagtgg gactagagac 360
cacagtcaga agggccaacc gtgtactaag tgatgcactc aagagatacc ctagaatgga 420
catcccaaaa attgccagat ccttctatga cttatttgaa caaaaagtgt atcacagaag 480
tttgttcatt gagtatggca aagcattagg ctcatcatct acaggcagca aagcagaaag 540
tctatttgtt aatatattca tgcaagctta tggagccggt caaacaatgc taaggttggg 600

ggtaattgcc aggtcatcca acaatataat gttaggacat gtatccgtcc aagctgagtt 660
aaaacaggtc acagaagtct atgacttggt gcgagaaatg 700

<210> 11
<211> 705
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(705)
<223> human metapneumovirus, strain Quebec Isolate No hMPV-174

<400> 11
tattagcaca gctttaggat cagagagagt gcagcagatt ctgaggaact caggcagtga 60
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agatttacag atgttagaca tacacggggt agagaagagc tgggtagaag agatagacaa 180
agaagcaagg aaaacaatgg caaccttgct taaggaatca tcaggtaata tcccgcaaaa 240
tcagaggccc tcagcaccag acacacccat aatcttatta tgtgtaggtg ccttaatatt 300
tactaaacta gcatcaacca tagaagtggg actagagacc acagtcagaa gggctaaccg 360
tgtactaagt gatgcactca agagataccc tagaatggac atcccaaaaa ttgccagatc 420
cttctatgac ttatttgaac aaaaagtgta tcacagaagt ttgttcattg agtatggcaa 480
agcattaggc tcatcatcta caggcagcaa agcagaaagt gtatttgtta atatattcat 540
gcaagcttat ggagccggtc aaacaatgct gaggtggggg gtcattgcca ggtcatccaa 600
caatataatg ttaggacatg tatccgtcct agctgagcta aaaccggtca cagaagtcta 660
tgacttggtg cgagaaatga ttcctgaatc tngacttcta catta 705

<210> 12
<211> 700
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(700)
<223> human metapneumovirus, strain Quebec Isolate No hMPV-184

<400> 12
aaatatgctg cagaaatagg aatacaatat attagcacag ctttaggatc agagagagtg 60
cagcagattc tgaggaactc aggcagtgaa gtccaagtgg tcttaaccag aacgtactct 120
ctggggaaag ttaaaaacaa taaaggagaa gatttacaga tgttagacat acacggggta 180
gagaagagct gggtagaaga gatagacaaa gaagcaagga aaacaatggc aaccttgctt 240
aaggaatcat caggtaatat cccacaaaat cagaggccct cagcaccaga cacacccata 300
atcttattat gtgtaggtgc cttaatattt actaaactag catcaaccat agaagtggga 360
ctagagacca cagtcagaag ggccaaccgt gtactaagtg atgcactcaa gagatacccc 420
agaatggaca tcccaaaaat tgccagatcc ttctatgact tatttgaaca aaaagtgtat 480
cacagaagtt tgttcattga gtatggcaaa gcattaggct catcatctac aggcagcaaa 540
gcagaaagtc tatttgttaa tatattcatg caagcttatg gagccggtca aacaatgcta 600
aggtgggggg taattgccag atcatccaac aatataatgt taggacatgt atccgtccaa 660
gctgagttaa aacaggtcac agaagtctat gacttagtgc 700

<210> 13
<211> 650
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(650)
<223> human metapneumovirus, strain Quebec Isolate No hMPV-185

<400> 13
taggaataca atatattagc acagctctag gatcagagag agtacagcag attctaagaa 60
actcaggcag tgaagtccaa gtggttttaa ccagaacgta ctccttgggg aaagttaaaa 120
acaacaaagg agaagattta cagatgttag acatacacgg agtagagaaa agctgggtgg 180
aagagataga caaagaagca agaaaaacaa tggcaacttt gcttaaagaa tcatcaggca 240
atattccaca aaatcagagg ccttcagcac cagacacacc tataatctta ttatgtgtag 300
gtgccttaat atttaccaaa ctagcatcaa ctatagaagt gggattagag accacagtca 360
gaagagctaa ccgtgtacta agtgatgcac tcaaaagata ccctaggatg gacataccaa 420

aaatcgctag atctttctat gacttatttg aacaaaaagt gtattacaga agcttgttca 480
ttgagtatgg caaagcatta ggctcatcct ctacaggcag caaagcagaa agtttattcg 540
ttaatatatt catgcaagct tacggtgctg gtcaaacaat gctgaggtgg ggagtcattg 600
ccaggtcatc taacaatata atgttaggac atgtatctgt ccaagctgag 650

<210> 14
<211> 650
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(650)
<223> human metapneumovirus, strain Quebec Isolate No C-76837

<400> 14
acaaatatgc atcataaata ggaatacaat atattagcac agctttagag atcagagaga 60
gtgcagcaga ttctaaggaa ctcaggcagt gaagtccaag tggtcttaac cagaacgtac 120
tctctgggga aagttaaaaa caataaagga gaagatttac agatgttaga catacacggg 180
gtagagaaga gctgggtaga agagatagac aaagaagcaa ggaaaacaat ggcaaccttg 240
cttaaggaat catcaggtaa tatcccacaa aatcagaggc cctcagcacc agacacaccc 300
ataatcttat tatgtgtagg tgccttaata tttactaaac tagcatcaac catagaagtg 360
ggactagaga ccacagtcag aagggctaac cgtgtactaa gtgatgcact caagagatac 420
cctagaatgg acatcccaaa aattgccaga tccttctatg acttatttga acaaaaagtg 480
tatcacagaa gtttgttcat tgagtatggc aaagcattag gctcatcatc tacaggcagc 540
aaagcagaaa gtctatttgt taatatattc atgcaagctt atggagccgg tcaaacaatg 600
ctaaggtggg gggtcattgc caggtcatcc aacaatataa tgttaggata 650

<210> 15
<211> 650
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(650)
<223> human metapneumovirus, strain Quebec Isolate No C-77807

<400> 15
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agctttagga tcagagagag tgcagcagat tctgaggaac tcaggcagtg aagtccaagt 120
ggtcttaacc agaacgtact ctctggggaa agttaaaaac aataaaagga gaagatttac 180
agatgttaga catacacggg gtagagaaga gctgggtaga agagatagac aaagaagcaa 240
ggaaaacaat ggcaaccttg cttaaggaat catcaggtaa tatcccacaa aatcagaggc 300
cctcagcacc agacacaccc ataatcttat tatgtgtagg tgccttaata tttactaagc 360
tagcatcaac catagaagtg ggactagaga ccacagtcag aagggctaac cgtgtactaa 420
gtgatgcact caagagatac cctagaatgg acatcccaaa aattgccaga tccttctatg 480
acttatttga acaaaaagtg tatcacagaa gtttgttcat tgagtatggc aaagcattag 540
gctcatcatc tacaggcagc aaagcagaaa gtctatttgt taatatattc atgcaagctt 600
atggagccgg tcaaacaatg ctaaggtggg gggtcattgc caggtcatcc 650

<210> 16
<211> 650
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(650)
<223> human metapneumovirus, strain Quebec Isolate No hMPV-C-78123

<400> 16
ttaggatcag agagagtgca gcagattcta aggaactcag gcagtgaagt ccaagtggtc 60
ttaaccagaa cgtactctct ggggaaagtt aaaaacaata aaggagaaga tttacagatg 120
ttagacatac acggggtaga gaagagctgg gtagaagaga tagacaaaga agcaaggaaa 180
acaatggcaa ccttgcttaa ggaatcatca ggtaatatcc cacaaaatca gaggccctca 240
gcaccagaca cacccataat cttattatgt gtaggtgcct taatatttac taaactagca 300
tcaaccatag aagtgggact agagaccaca gtcagaaggg ctaaccgtgt actaagtgat 360
gcactcaaga gataccctag aatggacatc ccaaaaattg ccagatcctt ctatgactta 420

tttgaacaaa aagtgtatca cagaagtttg ttcattgagt atggcaaagc attaggctca 480
tcatctacag gcagcaaagc agaaagtcta tttgttaata tattcatgca agcttatgga 540
gccggtcaaa caatgctaag gtgggggggtc attgccaggt catccaacaa tataatgtta 600
ggacatgtat ccgtccaagc tgagttaaaa caggtcacag aagtctatga 650

<210> 17
<211> 650
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(700)
<223> human metapneumovirus, strain Quebec Isolate No C-78132

<400> 17
cagaaagagt acaacagatt ttgagaaact caggtagtga agttcaggtg gttttaacca 60
aaacatactc cttagggaaa ggcaaaaaca gtaaagggga agagctgcag atgttagata 120
tacatggagt ggaaaagagt tgggtagaag aaatagacaa agaggcaaga aagacaatgg 180
taactttgct taaggagtca tcaggtaaca tcccacaaaa ccagagacct tcagcaccag 240
acacaccaat aattttatta tgtgtaggtg ccctaatatt cactaaacta gcatctacaa 300
tagaagttgg attagagact acagttagaa gagctaatag agtgctaagt gatgcactca 360
aaagataccc tagggtagat ataccaaaga ttgctagatc tttttatgaa ctatttgaac 420
aaaaagtgta ctacagaagt ttattcattg agtacggaaa agctttaggc tcatcttcaa 480
caggaagcaa agcagaaagt ttgtttgtaa atatatttat gcaagcttat ggagctggtc 540
aaacactgct aaggtggggt gtcattgcca gatcatccaa caacataatg ctagggcatg 600
tatctgtgca atctgaattg aagcaagtta cagaggttta tgacttggtg 650

<210> 18
<211> 650
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(650)
<223> human metapneumovirus, strain Quebec Isolate No C-78163

<400> 18
ggatcagaaa gagtacaaca gattttgaga aactcaggta gtgaagttca ggtggtttta 60
accaaaacat actccttagg gaaaggcaaa aacagtaaag gggaagagct gcagatgtta 120
gatatacatg gagtggaaaa gagttgggta gaagaaatag acaaagaggc aagaaagaca 180
atggtaactt tgcttaagga gtcatcaggt aacatcccac aaaaccagag accttcagca 240
ccagacacac caataatttt attatgtgta ggtgccctaa tattcactaa actagcatca 300
acaatagaag ttggattaga gactacagtt agaagagcta atagagtgct aagtgatgca 360
ctcaaaagat accctagggt agatatacca aagattgcta gatcttttta tgaactattt 420
gaacaaaaag tgtactacag aagtttattc attgagtacg gaaaagcttt aggctcatct 480
tcaacaggaa gcaaagcaga aagtttgttt gtaaatatat ttatgcaagc ttatggagct 540
ggtcaaacac tgctaaggtg gggtgtcatt gccagatcat ccaacaacat aatgctaggg 600
catgtatctg tgcaatctga attgaagcaa gttacagagg tttatgactt 650

<210> 19
<211> 600
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(600)
<223> human metapneumovirus, strain Quebec Isolate No C-91205

<400> 19
atattagcac agctctaggg atcagagaga gtacagcaga ttctaagaaa ctcaggcagt 60
gaagtccaag tggttttaac cagaacgtac tccttgggga aagttaaaaa caacaaagga 120
gaagatttac agatgttaga catacacgga gtagagaaaa gctgggtgga agagatagac 180
aaagaagcaa gaaaaacaat ggcaactttg cttaaagaat catcaggcaa tattccacaa 240
aatcagaggc cttcagcacc agacacacct ataatcttat tatgtgtagg tgccttaata 300
tttaccaaac tagcatcaac tatagaagtg ggattagaga ccacagtcag aagagctaac 360
cgtgtactaa gtgatgcact caaaagatac cctaggatgg acataccaaa aatcgctaga 420

tctttctatg acttatttga acaaaaagtg tattacagaa gcttgttcat tgagtatggc 480
aaagcattag gctcatcctc tacaggcagc aaagcagaaa gtttattcgt taatatattc 540
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<210> 20
<211> 700
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(700)
<223> human metapneumovirus, strain Quebec Isolate No C-91364

<400> 20
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agagtacaac agattttgag aaactcaggt agtgaagttc aggtggttct aaccaaaaca 120
tactccttag ggaaaggcaa aaacagtaaa ggggaagagc tgcagatgtt agatatacat 180
ggagtggaaa agagttgggt agaagaaata gacaaagagg caagaaagac aatggtaact 240
ttgcttaagg agtcatcagg taacatccca caaaaccaga gaccttcagc accagacaca 300
ccaataattt tattatgtgt aggtgcccta atattcacta aactagcatc aacaatagaa 360
gttggattag agactacagt tagaagagct aatagagtgc taagtgatgc actcaaaaga 420
taccctaggg tagatatacc aaagattgct agatcttttt atgaactatt tgaacaaaaa 480
gtgtactaca gaagtttatt cattgagtac ggaaaagctt taggctcatc ttcaacagga 540
agcaaagcag aaagtttgtt tgtaaatata tttatgcaag cttatggagc tggtcaaaca 600
ctgctaaggt ggggtgtcat tgccagatca tccaacaaca taatgctagg gcatgtatct 660
gtgcaatctg aattgaagca agttacagag gtttatgact 700

<210> 21
<211> 600
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(600)
<223> human metapneumovirus, strain quebec Isolate No C-91830

<400> 21
cagctctagg atcagaaaga gtacaacaga ttttgagaaa ctcaggtagt gaagttcagg 60
tggttctaac caaaacatac tccttaggga aaggcaaaaa cagtaaaggg gaagagctgc 120
agatgttaga tatacatgga gtggaaaaga gttgggtaga agaaatagac aaagaggcaa 180
gaaagacaat ggtaactttg cttaaggagt catcaggtaa catcccacaa aaccagagac 240
cttcagcacc agacacacca ataatttttat tatgtgtagg tgccttaata ttcactaaac 300
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gtgatgcact caaaagatac cctagggtag atataccaaa gattgctaga tctttttatg 420
aactatttga acaaaaagtg tactacagaa gcttattcat tgagtacgga aaagctttag 480
gctcatcttc aacaggaagc aaagcagaga gtttgtttgt aaatatattt atgcaagctt 540
atggagctgg tcaaacactg ctaaggtggg gtgtcattgc cagatcatcc aacaacataa 600

<210> 22
<211> 650
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(650)
<223> human metapneumovirus, strain Quebec Isolate No hMPV-Q86

<400> 22
atgctgatta caaatatgct gcagaaatag gaatacaata tattagcaca gctctagggt 60
cagagaaagt acagcagatt ctaagaaact caggcagtga agtccaagcg gttttaacca 120
gaacgtactc tttggggaaa gttaaaaaca ataaaggaga agatttacag atgttagaca 180
tacacggagt agaaaaaagc tgggtggaag agatagacaa agaagcaaga aaaacaatgg 240
caactttact taaagaatca tcaggtaata ttccacaaaa tcagaggcct tcagcaccag 300
acacacctat aatcttatta tgtgtaggtg ccttaatatt caccaaacta gcatcaacta 360
tagaagtggg attagagacc acagtcagaa gagctaaccg tgtactaagt gatgcactca 420
aaagataccc tagaatggac ataccaaaaa ttgctagatc cttctatgac ttatttgaac 480

aaaaagtgta tcacagaagt ttgttcattg agtatgggca aagcattagg ctcatcctct 540
acaggcagca aagcagaaag tttattcgtc aatatattca tgcaagctta tggtgctggt 600
caaacaatgc tgaggtgggg gggttatcgc caggtcatct aacaatataa 650

<210> 23
<211> 650
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(650)
<223> human metapneumovirus, strain Quebec Isolate No hMPV-Q97

<400> 23
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ccaagtggtt ttaaccagaa cgtactcctt ggggaaagtt aaaaacaaca aaggagaaga 120
tttacagatg ttagacatac acggagtaga gaaaagctgg gtggaagaga tagacaaaga 180
agcaagaaaa acaatggcaa ctttgcttaa agaatcatca ggcaatattc cacaaaatca 240
gaggccttca gcaccagaca cacctataat cttattatgt gtaggtgcct taatatttac 300
caaactagca tcaactatag aagtgggatt agagaccaca gtcagaagag ctaaccgtgt 360
actaagtgat gcactcaaaa gataccctag gatggacata ccaaaaatcg ctagatcttt 420
ctatgactta tttgaacaaa aagtgtatta cagaagcttg ttcattgagt atggcaaagc 480
attaggctca tcctctacag gcagcaaagc agaaagtttt attcgttaat atattcatgc 540
aagcttacgg tgctggtcaa acaatgctga ggtggggagt cattgccagg tcatctaaca 600
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<210> 24
<211> 741
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(741)
<223> human metapneumovirus, strain Can 97-83

<400> 24
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taagcatgct gattacaaat atgctgcaga aataggaata caatatatta gcacagctct 120
aggatcagag agagtacagc agattctaag aaactcaggc agtgaagtcc aagtggtttt 180
aaccagaacg tactccttgg ggaaagttaa aaacaacaaa ggagaagatt tacagatgtt 240
agacatacac ggagtagaga aaagctgggt ggaagagata gacaaagaag caagaaaaac 300
aatggcaact ttgcttaaag aatcatcagg caatattcca caaaatcaga ggccttcagc 360
accagacaca cctataatct tattatgtgt aggtgcctta atatttacca aactagcatc 420
aactatagaa gtgggattag agaccacagt cagaagagct aaccgtgtac taagtgatgc 480
actcaaaaga taccctagga tggacatacc aaaaatcgct agatctttct atgatttatt 540
tgaacaaaaa gtgtattaca gaagtttgtt cattgagtat ggcaaagcat taggctcatc 600
ctctacaggc agcaaagcag aaagtttatt cgttaatata ttcatgcaag cttacggtgc 660
tggtcaaaca atgctgaggt ggggagtcat tgccaggtca tctaacaata taatgttagg 720
acatgtatct gtccaagctg a 741

<210> 25
<211> 650
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(650)
<223> human metapneumovirus, strain Can 98-75

<400> 25
caatatattt gcacggctct aggatcagaa agagtacaac agattttaag aaattcaggc 60
agtgaagttc aggtggttct aaccaagaca tactctttag ggaaaggtaa aaatagtaaa 120
ggggaagagt tgcaaatgtt agatatacat ggagtggaaa agagttgggt agaagaaata 180
gacaaagagg caagaaaaac aatggtgact ttgctaaagg aatcatcagg taacatccca 240
caaaaccaga ggccttcagc accagacaca ccaataattt tattatgtgt aggtgcttta 300
atattcacta aactagcatc aacaatagaa gttggactag agactacagt tagaagagct 360
aacagagtgc taagtgatgc gctcaaaaga taccctaggg tagatatacc gaagattgct 420
agatctttct atgaactatt tgagcagaaa gtgtattaca ggagtctatt cattgagtat 480

gggaaagctt taggctcatc ttcaacagga agcaaagcag aaagtttgtt tgtaaatata 540
tttatgcaag cttatggagc cggtcaaaca atgctaaggt ggggtgtcat tgccagatca 600
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<210> 26
<211> 759
<212> DNA
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<220>
<221> gene
<222> (1)...(759)
<223> human metapneumovirus, strain Can 98-75

<400> 26
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acaggttggt acaccaatgt ctttacatta gaagttggtg atgttgaaaa tcttacatgt 180
actgatggac ctagcttaat caaaacagaa cttgacctaa ccaaaagtgc tctaagagaa 240
ctcaaaacag tttctgctga tcagttagcg agagaagaac aaattgaaaa tcccagacaa 300
tcaaggtttg tcctaggtgc aatagctctt ggtgttgcca cagcagcagc agtcacagca 360
ggcattgcga tagccaaaac cataaggctt gagagtgaag tgaatgcaat caaaggtgct 420
ctcaaaacaa ccaacgaggc agtatccaca ctaggaaatg gagtgcgagt cctagccacc 480
gcagtaagag agctgaaaga atttgtgagc aaaaacctga ctagtgcaat taacaagaac 540
aaatgtgaca ttgctgacct gaagatggct gtcagcttca gtcaattcaa cagaagattc 600
ctaaatgttg tgcggcagtt ttcagacaat gcagggataa caccagcaat atcattggac 660
ctaatgactg atgctgagct ggccagagct gtatcatata tgccaacatc tgcaggacag 720
ataaaactaa tgttagagaa ccgtgcaatg gtaagaaga 759

<210> 27
<211> 759
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(759)
<223> human metapneumovirus, strain Quebec Isolate No C-67345

<400> 27
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gagagctacc tagaagaatc atgtatcact ataactgagg gatatcttag tgttctgaga 120
acaggttggt ataccaacgt ttttacatta gaggtgggtg atgtagaaaa ccttacatgt 180
tctgatggac ctagcctaat aaaaacagaa ttagatctga ccaaaagtgc actaagagag 240
ctcaaaacag tctctgctga ccaattggca agagaggaac aaattgagaa tcccagacaa 300
tctaggtttg ttctgggagc aatagcactc ggtgttgcaa cagcagctgc agtcacagca 360
ggtgttgcaa ttgccaaaac catccggctt gagagtgaag tcacagcaat taagaatgcc 420
ctcaaaacga ccaatgaagc agtatctaca ttggggaatg gagttcgagt gttggcaact 480
gcagtgagag agctgaaaga ctttgtgagc aagaatttaa ctcgtgcaat caacaaaaac 540
aagtgcgaca ttgatgacct aaaaatggcc gttagcttca gtcaattcaa cagaaggttt 600
ctaaatgttg tgcggcaatt ttcagacaat gctggaataa caccagcaat atctttggac 660
ttaatgacag atgctgaact agccagggcc gtttccaaca tgccgacatc tgcaggacaa 720
ataaaattga tgttggagaa ccgtgcgatg gtaagaaga 759

<210> 28
<211> 759
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(759)
<223> human metapneumovirus, strain Can 97-83

<400> 28
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ctgaggacag gttggtatac caacgttttt acattagagg tgggtgatgt agaaaacctt 180
acatgttctg atggacctag cctaataaaa acagaattag atctgaccaa aagtgcacta 240
agagagctca aaacagtctc tgctgaccaa ttggcaagag aggaacaaat tgagaatccc 300
agacaatcta ggtttgttct aggagcaata gcactcggtg ttgcaacagc agctgcagtc 360

acagcaggtg ttgcaattgc caaaaccatc cggcttgaga gtgaagtcac agcaattaag 420
aatgccctca aaacgaccaa tgaagcagta tctacattgg ggaatggagt tcgagtgttg 480
gcaactgcag tgagagagct gaaagacttt gtgagcaaga atttaactcg tgcaatcaac 540
aaaaacaagt gcgacattga tgacctaaaa atggccgtta gcttcagtca attcaacaga 600
aggtttctaa atgttgtgcg gcaatttttca gacaatgctg gaataacacc agcaatatct 660
ttggacttaa tgacagatgc tgaactagcc agggccgttt ctaacatgcc gacatctgca 720
ggacaaataa aattgatgtt ggagaaccgt gcgatggtg 759

<210> 29
<211> 759
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(759)
<223> human metapneumovirus, strain quebec Isolate No C-68661

<400> 29
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gagagctatt tagaagagtc atgtagcact ataactgaag gatatctcag tgttctgagg 120
acaggttggt ataccaacgt ttttacactg gaggtaggtg atgtagagaa ccttacatgt 180
gctgatggac ctagcttaat aaaaacagaa ttagacctga ccaaaagtgc actaagagag 240
ctcagaacag tttctgctga tcaactggca agagaggagc aaattgagaa tcccagacaa 300
tctagatttg ttctaggagc aatagcactc ggtgttgcaa cagcagctgc agttacagca 360
ggtgttgcaa ttgccaaaac catccggctt gaaagtgaag taacagcaat taagaatgcc 420
ctcaaaaaga ccaatgaagc agtatctaca ttggggaatg gagttcgagt gttggcaact 480
gcagtgaggg agctgaaaga ttttgtgagc aagaatctaa cacgtgcaat caacaaaaac 540
aagtgcgaca ttgctgacct gaaaatggcc gttagcttca gtcaattcaa cagaaggttt 600
ctaaatgttg tgcggcaatt ttcagacaat gctggaataa caccagcaat atccttggac 660
ttaatgacag atgctgaact agccagagct gtttccaaca tgccaacatc tgcaggacaa 720
ataaaactga tgttggagaa ccgtgcaatg gtaagaaga 759

<210> 30
<211> 759
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(759)
<223> human metapneumovirus, strain Quebec Isolate No C-73750

<400> 30
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gagagctatt tagaagagtc atgtagcact ataactgaag gatatctcag tgttctgagg 120
acaggttggt ataccaacgt ttttacactg gaggtaggtg atgtagagaa ccttacatgt 180
gctgatggac ctagcttaat aaaaacagaa ttagacctga ccaaaagtgc actaagagag 240
ctcagaacag tttctgctga tcaactggca agagaggagc aaattgagaa tcccagacaa 300
tctagatttg ttctaggagc aatagcactc ggtgttgcaa cagcagctgc agttacagca 360
ggtgttgcaa ttgccaaaac catccggctt gaaagtgaag taacagcaat taagaatgcc 420
ctcaaaaaga ccaatgaagc agtatctaca ttggggaatg gagttcgagt gttggcaact 480
gcagtgaggg aactgaaaga ttttgtgagc aagaatctaa cacgtgcaat caacaaaaac 540
aagtgcgaca ttgctgacct gaaaatggcc gttagcttca gtcaattcaa cagaaaggtt 600
ctaaatgttg tgcggcaatt ttcagacaat gctggaataa caccagcaat atccttggac 660
ttaatgacag atgctgaact agccagagct gtttccaaca tgccaacatc tgcaggacaa 720
ataaaactga tgttggagaa ccgtgcaatg gtaagaaga 759

<210> 31
<211> 759
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(759)
<223> human metapneumovirus, strain Quebec Isolate No C-74549

<400> 31

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acaggttggt ataccaacgt ttttacatta gaggtgggtg atgtagaaaa ccttacatgt 180
tctgatggac ctagcctaat aaaaacagaa ttagatctga ccaaaagtgc actaagagag 240
ctcaaaacag tctctgctga ccaattggca agagaggaac aaattgagaa tcccagacaa 300
tctaggtttg ttctaggagc aatagcactc ggtgttgcaa cagcagctgc agtcacagca 360
ggtgttgcaa ttgccaaaac catccggctt gagagtgaag tcacagcaat taagaatgcc 420
ctcaaaacga ccaatgaagc agtatctaca ttggggaatg gagttcgagt gttggcaact 480
gcagtgagag agctgaaaga ctttgtgagc aagaatttaa ctcgtgcaat caacaaaaac 540
aagtgcgaca ttgatgacct aaaaatggcc gttagcttca gtcaattcaa cagaaggttt 600
ctaaatgttg tgcggcaatt ttcagacaat gctggaataa caccagcaat atctttggac 660
ttaatgacag atgctgaact agccagggcc gtttctaaca tgccgacatc tgcaggacaa 720
ataaaattga tgttggagaa ccgcgcgatg gtaagaaga 759

<210> 32
<211> 759
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(759)
<223> human metapneumovirus, strain Quebec Isolate No C-74783

<400> 32
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gagagctacc tagaagaatc atgtagcact ataactgagg gatatcttag tgttctgagg 120
acaggttggt ataccaacgt ttttacatta gaggtgggtg atgtagaaaa ccttacatgt 180
tctgatggac ctagcctaat aaaaacagaa ttagatctga ccaaaagtgc actaagagag 240
ctcaaaacag tctctgctga ccaattggca agagaggaac aaattgagaa tcccagacaa 300
tctaggtttg ttctaggagc aatagcactc ggtgttgcaa cagcagctgc agtcacagca 360
ggtgttgcaa ttgccaaaac catccggctt gagagtgaag tcacagcaat taagaatgcc 420
ctcaaaacga ccaatgaagc agtatctaca ttggggaatg gagttcgagt gttggcaact 480
gcagtgagag agctgaaaga ctttgtgagc aagaatttaa ctcgtgcaat caacaaaaac 540
aagtgcgaca ttgatgacct aaaaatggcc gttagcttca gtcaattcaa cagaaggttt 600
ctaaatgttg tgcggcaatt ttcagacaat gctggaataa caccagcaat atctttggac 660
ttaatgacag atgctgaact agccagggcc gtttctaaca tgccgacatc tgcaggacaa 720
ataaaattga tgttggagaa ccgcgcgatg gtaagaaga 759

<210> 33
<211> 759
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(759)
<223> human metapneumovirus, strain Quebec Isolate No C-76837

<400> 33
atgtcttgga aagtggtgat cattttttca ttgttaataa cacctcaaca cggtcttaaa 60
gagagctatt tagaagagtc atgtagcact ataactgaag gatatctcag tgttctgagg 120
acaggttggt ataccaacgt ttttacactg gaggtaggtg atgtagagaa ccttacatgt 180
gctgatggac ctagcttaat aaaaacagaa ttagatctga ccaaaagtgc actaagagag 240
ctcagaacag tttctgctga tcaactggca agagaggagc aaattgagaa tcccagacaa 300
tctagatttg ttctaggagc aatagcactc ggtgttgcaa cagcagctgc agttacagca 360
ggtgttgcaa ttgccaaaac catccggctt gaaagtgaag taacagcaat taagaatgcc 420
ctcaaaaaga ccaatgaagc agtatctaca ttggggaatg gagttcgagt gttggcaact 480
gcagtgaggg aactgaaaga ttttgtgagc aagaatctaa cacgtgcaat caacaaaaac 540
aagtgcgaca ttgctgacct gaaaatggcc gttagcttca gtcaattcaa cagaaggttt 600
ctaaatgttg tgcggcaatt ttcagacaat gctggaataa cacccacaat atccttggac 660
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ataaaactga tgttggagaa ccgtgcaatg gtaagaaga 759

<210> 34
<211> 759
<212> DNA
<213> human metapneumovirus

<220>

<221> gene
<222> (1)...(759)
<223> human metapneumovirus, strain Quebec Isolate No C-77748

<400> 34
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gagagctatt tagaagagtc atgtagcact ataactgaag gatatctcag tgttctgagg 120
acaggttggt ataccaacgt ttttacactg aaggtaggtg atgtagagaa ccttacatgt 180
gctgatggac ctagcttaat aaaaacagaa ttagacctga ccaaaagtgc actaagagaa 240
ctcagaacag tttctgctga tcaactggca agagaggagc aaattgagaa tcccagacaa 300
tctagatttg ttctaggagc aatagcactc ggtgttgcaa cagcagctgc agttacagca 360
ggtgttgcaa ttgccaaaac catccggctt gaaagtgaag taacagcaat taagaatgcc 420
ctcaaaaaga ccaatgaagc agtatctaca ttggggaatg gagttcgagt gttggcaact 480
gcagtgaggg agctgaaaga ttttgtgagc aagaatctaa cacgtgcaat caacaaaaac 540
aagtgcgaca ttgctgacct gaaaatggcc gttagcttca gtcaattcaa cagaaggttt 600
ctaaatgttg tgcggcaatt ttcagacaat gctggaataa caccagcaat atccttggac 660
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ataaaactga tgttggagaa ccgtgcaatg gtaagaaga 759

<210> 35
<211> 759
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(759)
<223> human metapneumovirus, strain Quebec Isolate No C-77807

<400> 35
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gagagctatt tagaagagtc atgtagcact ataactgaag gatatctcag tgttctgagg 120
acaggttggt ataccaacgt ttttacactg aaggtaggtg atgtagagaa ccttacatgt 180
gctgatggac ctagcttaat aaaaacagaa ttagatctga ccaaaagtgc actaagagag 240
ctcagaccag tttctgctga tcaactggca agagaggagc aaattgagaa tcccagacaa 300
tctagatttg ttctaggagc aatagcactc ggtgttgcaa cagcagctgc agttacagca 360
ggtgttgcaa ttgccaaaac catccggctt gaaagtgaag taacagcaat taagaatgcc 420
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gcagtgaggg aactgaaaga ttttgtgagc aagaatctaa cacgtgcaat caacaaaaac 540
aagtgcgaca ttgctgacct gaaaatggcc gttagcttca gtcaattcaa cagaaggttt 600
ctaaatgttg tgcggcaatt ttcagacaat gctggaataa caccagcaat atccttggac 660
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ataaaactga tgttggagaa ccgtgcaatg gtaagaaga 759

<210> 36
<211> 759
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(759)
<223> human metapneumovirus, strain Quebec Isolate No C-78132

<400> 36
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gagagctatt tagaagagtc atgtagcact ataactgaag gatatctcag tgttctgagg 120
acaggttggt ataccaacgt ttttacactg gaggtaggtg atgtagagaa ccttacatgt 180
gctgatggac ctagcttaat aaaaacagaa ttagatctga ccaaaagtgc actaagagag 240
ctcagaacag tttctgctga tcaactggca agagaggagc aaattgagaa tcccagacaa 300
tctagatttg ttctaggagc aatagcactc ggtgttgcaa cagcagctgc agttacagca 360
ggtgttgcaa ttgccaaaac catccggctt gaaagtgaag taacagcaat taagaatgcc 420
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gcagtgaggg aactgaaaga ttttgtgagc aagaatctaa cacgtgcaat caacaaaaac 540
aagtgcgaca ttgctgacct gaaaatggcc gttagcttca gtcaattcaa cagaaggttt 600
ctaaatgttg tgcggcaatt ttcagacaat gctggaataa caccagcaat atccttggac 660
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ataaaactga tgttggagaa ccgtgcaatg gtaagaaga 759

<210> 37
<211> 759
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(759)
<223> human metapneumovirus, strain Quebec Isolate No C-78163

<400> 37
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gagagctatt tagaagagtc atgtagcact ataactgaag gatatctcag tgttctgagg 120
acaggttggt ataccaacgt ttttacactg gaggtaggtg atgtagagaa ccttacatgt 180
gctgatggac ctagcttaat aaaaacagaa ttagatctga ccaaaagtgc actaagagag 240
ctcagaacag tttctgctga tcaactggca agagaggagc aaattgagaa tcccagacaa 300
tctagatttg ttctaggagc aatagcactc ggtgttgcaa cagcagctgc agttacagca 360
ggtgttgcaa ttgccaaaac catccggctt gaaagtgaag taacagcaat taagaatgcc 420
ctcaaaaaga ccaatgaagc agtatctaca ttggggaatg gagttcgagt gttggcaact 480
gcagtgaggg aactgaaaga ttttgtgagc aagaatctaa cacgtgcaat caacaaaaac 540
aagtgcgaca ttgctgacct gaaaatggcc gttagcttca gtcaattcaa cagaaggttt 600
ctaaatgttg tgcggcaatt ttcagacaat gctggaataa caccagcaat atccttggac 660
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<210> 38
<211> 759
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(759)
<223> human metapneumovirus, strain Quebec Isolate No C-78406

<400> 38
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gagagctatt tagaagagtc atgtagcact ataactgaag gatatctcag tgttctgagg 120
acaggttggt ataccaacgt ttttacactg gaggtaggtg atgtagagaa ccttacatgt 180
gctgatggac ctagcttaat aaaaacagaa ttagatctga ccaaaagtgc actaagagag 240
ctcagaacag tttctgctga tcaactggca agagaggagc aaattgagaa tcccagacaa 300
tctagatttg ttctaggagc aatagcactc ggtgttgcaa cagcagctgc agttacagca 360
ggtgttgcaa ttgccaaaac catccggctt gaaagtgaag taacagcaat taagaatgcc 420
ctcaaaaaga ccaatgaagc agtatctaca ttggggaatg gagttcgagt gttggcaact 480
gcagtgaggg aactgaaaga ttttgtgagc aagaatctaa cacgtgcaat caacaaaaac 540
aagtgcgaca ttgctgacct gaaaatggcc gttagcttca gtcaattcaa cagaaggttt 600
ctaaatgttg tgcggcaatt ttcagacaat gctggaataa caccagcaat atccttggac 660
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ataaaactga tgttggagaa ccgtgcaatg gtaagaaga 759

<210> 39
<211> 759
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(759)
<223> human metapneumovirus, strain Quebec Isolate No hMPV-174

<400> 39
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gagagctatt tagaagagtc atgtagcact ataactgaag gatatctcag tgttctgagg 120
acaggttggt ataccaacgt ttttacactg gaggtaggtg atgtagagaa ccttacatgt 180
gctgatggac ctagcttaat aaaaacagaa ttagacctga ccaaaagtgc actaagagag 240
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tctagatttg ttctaggagc aatagcactc ggtgttgcaa cagcagctgc agttacagca 360
ggtgttgcaa ttgccaaaac catccggctt gaaagtgaag taacagcaat taagaatgcc 420
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gcagtgagag agctgaaaga ttttgtgagc aagaatctaa cacgtgcaat caacaaaaac 540
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ctaaatgttg tgcggcaatt ttcagacaat gctggaataa caccagcaat atccttggac 660
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ataaaactga tgttggagaa ccgtgcaatg gtaagaaga 759

<210> 40
<211> 759
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(759)
<223> human metapneumovirus, strain Quebec Isolate No hMPV-193

<400> 40
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gagagctatt tagaagagtc atgtagcact ataactgaag gatatctcag tgttctgagg 120
acaggttggt ataccaacgt ttttacactg gaggtaggtg atgtagagaa ccttacatgt 180
gctgatggac ctagcttaat aaaaacagaa ttagacctga ccaaaagtgc actaagagag 240
ctcagaacag tttctgctga tcaactggca agagaggagc aaattgagaa tcccagacaa 300
tctagatttg ttctaggagc aatagcactc ggtgttgcaa cagcagctgc agttacagca 360
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ctcaaaaaga ccaatgaagc agtatctaca ttggggaatg gagttcgagt gttggcaact 480
gcagtgagag agctgaaaga ttttgtgagc aagaatctaa cacgtgcaat caacaaaaac 540
aagtgcgaca ttgctgacct gaaaatggcc gttagcttca gtcaattcaa cagaaggttt 600
ctaaatgttg tgcggcaatt ttcagacaat gctggaataa caccagcaat atccttggac 660
ttaatgacag atgctgaact agccagagct gtttccaaca tgccaacatc tgcaggacaa 720
ataaaactga tgttggagaa ccgtgcaatg gtaagaaga 759

<210> 41
<211> 759
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(759)
<223> human metapneumovirus, strain Quebec Isolate No hMPV-194

<400> 41
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gagagctatt tagaagagtc atgtagcact ataactgaag gatatctcag tgttctgagg 120
acaggttggt ataccaacgt ttttacactg gaggtaggtg atgtagagaa ccttacatgt 180
gctgatggac ctagcttaat aaaaacagaa ttagacctga ccaaaagtgc actaagagag 240
ctcagaacag tttctgctga tcaactggca agagaggagc aaattgagaa tcccagacaa 300
tctagatttg ttctaggagc aatagcactc ggtgttgcaa cagcagctgc agttacagca 360
ggtgttgcaa ttgccaaaac catccggctt gaaagtgaag taacagcaat taagaatgcc 420
ctcaaaaaga ccaatgaagc agtatctaca ttggggaatg gagttcgagt gttggcaact 480
gcagtgagag agctgaaaga ttttgtgagc aagaatctaa cacgtgcaat caacaaaaac 540
aagtgcgaca ttgctgacct gaaaatggcc gttagcttca gtcaattcaa cagaaggttt 600
ctaaatgttg tgcggcaatt ttcagacaat gctggaataa caccagcaat atccttggac 660
ttaatgacag atgctgaact agccagagct gtttccaaca tgccaacatc tgcaggacaa 720
ataaaactga tgttggagaa ccgtgcaatg gtaagaaga 759

<210> 42
<211> 759
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(759)
<223> human metapneumovirus, strain Quebec Isolate No hMPV-198

<400> 42
atgtcttgga aagtggtgat catttttca ttgttaataa cacctcaaca cggtcttaaa 60
gagagctatt tagaagagtc atgtagcact ataactgaag gatatctcag tgttctgagg 120

acaggttggt ataccaacgt ttttacactg gaggtaggtg atgtagagaa ccttacatgt 180
gctgatggac ctagcttaat aaaaacagaa ttagacctga ccaaaagtgc actaagagag 240
ctcagaacag tttctgctga tcaactggca agagaggagc aaattgagaa tcccagacaa 300
tctagatttg ttctaggagc aatagcactc ggtgttgcaa cagcagctgc agttacagca 360
ggtgttgcaa ttgccaaaac catccggctt gaaagtgaag taacagcaat taagaatgcc 420
ctcaaaaaga ccaatgaagc agtatctaca ttggggaatg gagttcgagt gttggcaact 480
gcagtgagag agctgaaaga ttttgtgagc aagaatctaa cacgtgcaat caacaaaaac 540
aagtgcgaca ttgctgacct gaaaatggcc gttagcttca gtcaattcaa cagaaggttt 600
ctaaatgttg tgcggcaatt ttcagacaat gctggaataa caccagcaat atccttggac 660
ttaatgacag atgctgaact agccagagct gtttccaaca tgccaacatc tgcaggacaa 720
ataaaactga tgttggagaa ccgtgcaatg gtaagaaga 759

<210> 43
<211> 759
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(759)
<223> human metapneumovirus, strain Quebec Isolate No
hMPV-217

<400> 43
atgtcttgga aagtggtgat catttttta ttgttaataa cacctcaaca cggtcttaaa 60
gagagctatt tagaagagtc atgtagcact ataactgaag gatatctcag tgttctgagg 120
acaggttggt ataccaacgt ttttacactg gaggtaggtg atgtagagaa ccttacatgt 180
gctgatggac ctagcttaat aaaaacagaa ttagacctga ccaaaagtgc actaagagag 240
ctcagaacag tttctgctga tcaactggca agagaggagc aaattgagaa tcccagacaa 300
tctagatttg ttctaggagc aatagcactc ggtgttgcaa cagcagctgc agttacagca 360
ggtgttgcaa ttgccaaaac catccggctt gaaagtgaag taacagcaat taagaatgcc 420
ctcaaaaaga ccaatgaagc agtatctaca ttggggaatg gagttcgagt gttggcaact 480
gcagtgagag agctgaaaga ttttgtgagc aagaatctaa cacgtgcaat caacaaaaac 540
aagtgcgaca ttgctgacct gaaaatggcc gttagcttca gtcaattcaa cagaaggttt 600
ctaaatgttg tgcggcaatt ttcagacaat gctggaataa caccagcaat atccttggac 660
ttaatgacag atgctgaact agccagagct gtttccaaca tgccaacatc tgcaggacaa 720
ataaaactga tgttggagaa ccgtgcaatg gtaagaaga 759

<210> 44
<211> 759
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(759)
<223> human metapneumovirus, strain Quebec Isolate No
hMPV-208

<400> 44
atgtcttgga aagtggtgat catttttta ttgttaataa cacctcaaca cggtcttaaa 60
gagagctatt tagaagagtc atgtagcact ataactgaag gatatctcag tgttctgagg 120
acaggttggt ataccaacgt ttttacactg gaggtaggtg atgtagagaa ccttacatgt 180
gctgatggac ctagcttaat aaaaacagaa ttagacctga ccaaaagtgc actaagagag 240
ctcagaacag tttctgctga tcaactggca agagaggagc aaattgagaa tcccagacaa 300
tctagatttg ttctaggagc aatagcactc ggtgttgcaa cagcagctgc agttacagca 360
ggtgttgcaa ttgccaaaac catccggctt gaaagtgaag taacagcaat taagaatgcc 420
ctcaaaaaga ccaatgaagc agtatctaca ttggggaatg gagttcgagt gttggcaact 480
gcagtgaggg agctgaaaga ttttgtgagc aagaatctaa cacgtgcaat caacaaaaac 540
aagtgcgaca ttgctgacct gaaaatggcc gttagcttca gtcaattcaa cagaaggttt 600
ctaaatgttg tgcggcaatt ttcagacaat gctggaataa caccagcaat atccttggac 660
ttaatgacag atgctgaatt agccagagct gtttccaaca tgccaacatc tgcaggacaa 720
ataaaactga tgttggagaa ccgtgcaatg gtaagaaga 759

<210> 45
<211> 759
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(759)

<223> human metapneumovirus, strain Quebec Isolate No hMPV-223

<400> 45
atgtcttgga aagtggtgat catttttca ttgttaataa cacctcaaca cggtcttaaa 60
gagagctatt tagaagagtc atgtagcact ataactgaag gatatctcag tgttctgagg 120
acaggttggt ataccaacgt ttttacactg gaggtaggtg atgtagagaa ccttacatgt 180
gctgatggac ctagcttaat aaaaacagaa ttagacctga ccaaaagtgc actaagagag 240
ctcagaacag tttctgctga tcaactggca agagaggagc aaattgagaa tcccagacaa 300
tctagatttg ttctaggagc aatagcactc ggtgttgcaa cagcagctgc agttacagca 360
ggtgttgcaa ttgccaaaac catccggctt gaaagtgaag taacagcaat taagaatgcc 420
ctcaaaaaga ccaatgaagc agtatctaca ttggggaatg gagttcgagt gttggcaact 480
gcagtgagag agctgaaaga ttttgtgagc aagaatctaa cacgtgcaat caacaaaaac 540
aagtgcgaca ttgctgacct gaaaatggcc gttagcttca gtcaattcaa cagaaggttt 600
ctaaatgttg tgcggcaatt ttcagacaat gctggaataa caccagcaat atccttggac 660
ttaatgacag atgctgaact agccagagct gtttccaaca tgccaacatc tgcaggacaa 720
ataaaactga tgttggagaa ccgtgcaatg gtaagaaga 759

<210> 46
<211> 759
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(759)
<223> human metapneumovirus, strain Quebec Isolate No hMPV-228

<400> 46
atgtcttgga aagtggtgat catttttca ttgttaataa cacctcaaca cggtcttaaa 60
gagagctatt tagaagagtc atgtagcact ataactgaag gatatctcag tgttctgagg 120
acaggttggt ataccaacgt ttttacactg gaggtaggtg atgtagagaa ccttacatgt 180
gctgatggac ctagcttaat aaaaacagaa ttagacctga ccaaaagtgc actaagagag 240
ctcagaacag tttctgctga tcaactggca agagaggagc aaattgagaa tcccagacaa 300
tctagatttg ttctaggagc aatagcactc ggtgttgcaa cagcagctgc agttacagca 360
ggtgttgcaa ttgccaaaac catccggctt gaaagtgaag taacagcaat taagaatgcc 420
ctcaaaaaga ccaatgaagc agtatctaca ttggggaatg gagttcgagt gttggcaact 480
gcagtgagag agctgaaaga ttttgtgagc aagaatctaa cacgtgcaat caacaaaaac 540
aagtgcgaca ttgctgacct gaaaatggcc gttagcttca gtcaattcaa cagaaggttt 600
ctaaatgttg tgcggcaatt ttcagacaat gctggaataa caccagcaat atccttggac 660
ttaatgacag atgctgaact agccagagct gtttccaaca tgccaacatc tgcaggacaa 720
ataaaactga tgttggagaa ccgtgcaatg gtaagaaga 759

<210> 47
<211> 759
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(759)
<223> human metapneumovirus, strain Quebec Isolate No C-85885

<400> 47
atgtcttgga aagtggtgat catttttca ttgttaataa cacctcaaca cggtcttaaa 60
gagagctatt tagaagagtc atgtagcact ataactgaag gatatctcag tgttctgagg 120
acaggttggt ataccaacgt ttttacactg gaggtaggtg atgtagagaa ccttacatgt 180
gctgatggac ctagcttaat aaaaacagaa ttagacctga ccaaaagtgc actaagagag 240
ctcagaacag tttctgctga tcaactggca agagaggagc aaattgagaa tcccagacaa 300
tctagatttg ttctaggagc aatagcactc ggtgttgcaa cagcagctgc agttacagca 360
ggtgttgcaa ttgccaaaac catccggctt gaaagtgaag taacagcaat taagaatgcc 420
ctcaaaaaga ccaatgaagc agtatctaca ttggggaatg gagttcgagt gttggcaact 480
gcagtgagag agctgaaaga ttttgtgagc aagaatctaa cacgtgcaat caacaaaaac 540
aagtgcgaca ttgctgacct gaaaatggcc gttagcttca gtcaattcaa cagaaggttt 600
ctaaatgttg tgcggcaatt ttcagacaat gctggaataa caccagcaat atccttggac 660
ttaatgacag atgctgaact agccagagct gtttccaaca tgccaacatc tgcaggacaa 720
ataaaactga tgttggagaa ccgtgcaatg gtaagaaga 759

<210> 48
<211> 759

<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(759)
<223> human metapneumovirus, strain Quebec Isolate No C-60298

<400> 48
atgtcttgga aagtggtgat catttttca ttgttaataa cacctcaaca cggtcttaaa 60
gagagctatt tagaagagtc atgtagcact ataactgaag gatatctcag tgttctgagg 120
acaggttggt ataccaacgt ttttacactg gaggtaggtg atgtagagaa ccttacatgt 180
gctgatggac ctagcttaat aaaaacagaa ttagatctga ccaaaagtgc actaagagag 240
ctcagaacag tttctgctga tcaactggca agagaggagc aaattgagaa tcccagacaa 300
tctagatttg ttctaggagc aatagcactc ggtgttgcaa cagcagctgc agttacagca 360
ggtgttgcaa ttgccaaaac catccggctt gaaagtgaag taacagcaat taagaatgcc 420
ctcaaaaaga ccaatgaagc agtatctaca ttggggaatg gagttcgagt gttggcaact 480
gcagtgaggg aactgaaaga ttttgtgagc aagaatctaa cacgtgcaat caacaaaaac 540
aagtgcgaca ttgctgacct gaaaatggcc gttagcttca gtcaattcaa cagaaggttt 600
ctaaatgttg tgcggcaatt ttcagacaat gctggaataa caccagcaat atccttggac 660
ttaatgacag atgctgaact agccagagct gtttccaaca tgccaacatc tgcaggacaa 720
ataaaactga tgttggagaa ccgtgcaatg gtaagaaga 759

<210> 49
<211> 759
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(759)
<223> human metapneumovirus, strain Quebec Isolate No C-63778

<400> 49
atgtcttgga aagtggtgat tatcatttcg ttactcataa cacctcagca cggactaaag 60
gaaagttatt tagaagaatc atgtagtact ataactgaag gatatctcag tgttttaaga 120
acaggttggt acaccaatgt ctttacatta gaagttggtg atgttgaaaa tcttacatgt 180
actgatggac ctagcttaat caaaacagaa cttgacctaa ccaaaagtgc tctaagagaa 240
ctcaaaacag tttctgctga tcagttagcg agagaagaac aaattgaaaa tcccagacaa 300
tcaaggtttg tcctaggtgc aatagctctt ggtgttgcca cagcagcagc agtcacagca 360
ggcattgcga tagccaaaac cataaggctt gagagtgaag tgaatgcaat caaaggtgct 420
ctcaaaacaa ccaacgaggc agtatccaca ctaggaaatg gagtgcgagt cctagccacc 480
gcagtaagag agctgaaaga atttgtgagc aaaaacctga ctagtgcaat taacaagaac 540
aaatgtgaca ttgctgatct gaagatggct gtcagcttca gtcaattcaa cagaagattc 600
ctaaatgttg tgcggcagtt ttcagacaat gcagggataa caccagcaat atcattggac 660
ctaatgactg atgctgagct ggccagaact gtatcataca tgccaacatt tgcaggacag 720
ataaaactaa tgttagagaa ccgtgcaatg gtaagaaga 759

<210> 50
<211> 759
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(759)
<223> human metapneumovirus, strain Quebec Isolate No C-64199

<400> 50
atgtcttgga aagtggtgat tatcatttcg ttactcataa cacttcagca cggactaaaa 60
gaaagttatt tagaagaatc atgtagtact ataactgaag gatatctcag tgttttaaga 120
acaggttggt acaccaatgt ctttacatta gaagttggtg atgttgaaaa tcttacatgt 180
actgatggac ctagcttaat caaaacagaa cttgacctaa ccaaaagtgc tctaagagaa 240
ctcaaaacag tttctgctga tcagttagcg agagaagaac aaattgaaaa tcccagacaa 300
tcaaggtttg tcctaggtgc aatagctctt ggtgttgcca cagcagcagc agtcacagca 360
ggcattgcga tagccaaaac cataaggctt gagagtgaag tgaatgcaat caaagatgct 420
ctcaaaacaa ccaacgaggc agtatccaca ctaggaaatg gagtgcgagt cctagccacc 480
gcagtaagag agctgaaaga atttgtgagc aaaaacctga ctagtgcaat taacaagaac 540
aaatgtgaca ttgctgatct gaagatggct gtcagcttca gtcaattcaa cagaagattc 600

ctaaatgttg tgcggcagtt ttcagacaat gcagggataa caccagcaat atcattggac 660
ctaatgactg atgctgagct ggccagagct gtatcataca tgccaacatc tgcaggacag 720
ataaaactaa tgttagagaa ccgtgcaatg gtaagaaga 759

<210> 51
<211> 759
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(759)
<223> human metapneumovirus, strain Quebec Isolate No C-64200

<400> 51
atgtcttgga aagtggtgat tatcatttcg ttactcataa cacctcagca cggactaaaa 60
gaaagttatt tagaagaatc atgtagtact ataactgaag gatatctcag tgttttaaga 120
acaggttggt acaccaatgt ctttacatta gaagttggtg atgttgaaaa tcttacatgt 180
actgatggac ctagcttaat caaaacagaa cttgacctaa ccaaaagtgc tctaagagaa 240
ctcaaaacag tttctgctga tcagttagcg agagaagaac aaattgaaaa tcccagacaa 300
tcaaggtttg tcctaggtgc aatagctctt ggtgttgcca cagcagcagc agtcacagca 360
ggcattgcga tagccaaaac cataaggctt gagagtgaag tgaatgcaat caaaggtgct 420
ctcaaaacaa ccaacgaggc agtatccaca ctaggaaatg gagtgcgagt cctagccacc 480
gcagtaagag agctgaaaga atttgtgagc aaaaacctga ctagtgcaat taacaagaac 540
aaatgtgaca ttgctgatct gaagatggct gtcagcttca gtcaattcaa cagaagattc 600
ctaaatgttg tgcggcagtt ttcagacaat gcagggataa caccagcaat atcattggac 660
ctaatgactg atgctgagct ggccagagct gtatcataca tgccaacatc tgcaggacag 720
ataaaactaa tgttagagaa ccgtgcaatg gtaagaaga 759

<210> 52
<211> 759
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(759)
<223> human metapneumovirus, strain Quebec Isolate No C-64536

<400> 52
atgtcttgga aagtggtgat tatcatttcg ttactcataa cacctcagca cggactaaaa 60
gaaagttatt tagaagaatc atgtagtact ataactgaag gatatctcag tgttttaaga 120
acaggttggt acaccaatgt ctttacatta gaagttggtg atgttgaaaa tcttacatgt 180
actgatggac ctagcttaat caaaacagaa cttgacctaa ccaaaagtgc tctaagagaa 240
ctcaaaacag tttctgctga tcagttagcg agagaagaac aaattgaaaa tcccagacaa 300
tcaaggtttg tcctaggtgc aatagctctt ggtgttgcca cagcagcagc agtcacagca 360
ggcattgcga tagccaaaac cataaggctt gagagtgaag tgaatgcaat caaaggtgct 420
ctcaaaacaa ccaacgaggc agtatccaca ctaggaaatg gagtgcgagt cctagccacc 480
gcagtaagag agctgaaaga atttgtgagc aaaaacctga ctagtgcaat taacaagaac 540
aaatgtgaca ttgctgatct gaagatggct gtcagcttca gtcaattcaa cagaagattc 600
ctaaatgttg tgcggcagtt ttcagacaat gcagggataa caccagcaat atcattggac 660
ctaatgactg atgctgagct ggccagagct gtatcataca tgccaacatc tgcaggacag 720
ataaaactaa tgttagagaa ccgtgcaatg gtaagaaga 759

<210> 53
<211> 759
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(759)
<223> human metapneumovirus, strain Quebec Isolate No C-73769

<400> 53
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gaaagttatt tagaagaatc atgtagtact ataactgaag gatatctcag tgttttaaga 120
ccaggttggt acaccaatgt ctttacatta gaagttggtg atgttgaaaa tcttacatgt 180
actgatggac ctagcttaat caaaacagaa cttgacctaa ccaaaagtgc tctaagagaa 240

ctcaaaacag tttctgctga tcagttagcg agagaagaac aaattgaaaa tcccagacaa 300
tcaaggtttg tcctaggtgc aatagctctt ggtgttgcca cagcagcagc agtcacagca 360
ggcattgcga tagccaaaac cataaggctt gagagtgaag tgaatgcaat caaaggtgct 420
ctcaaaacaa ccaacgaggc agtatccaca ctaggaaatg gagtgcgagt cctagccacc 480
gcagtaagag agttgaaaga atttgtgagc aaaaacctga ctagtgcaat taacaagaac 540
aaatgtgaca ttgctgatct gaagatggct gtcagcttca gtcaattcaa cagaagattc 600
ctaaatgttg tgcggcagtt ttcagacaat gcagggataa caccagcaat atcattagac 660
ctaatgactg atgctgagct ggccagagct gtatcataca tgccaacatc tgcaggacag 720
ataaaactaa tgttagagaa ccgtgcaatg gtaagaaga 759

<210> 54
<211> 759
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(759)
<223> human metapneumovirus, strain Quebec Isolate No C-74501

<400> 54
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gaaagttatt tagaagaatc atgtagtact ataactgaag gatatctcag tgttttaaga 120
acaggttggt acaccaatgt ctttacatta gaagttggtg atgttgaaaa tcttacatgt 180
actgatggac ctagcttaat caaaacagaa cttgacctaa ccaaaagtgc tctaagagaa 240
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tcaaggtttg tcctaggtgc aatagctctt ggtgttgcca cagcagcagc agtcacagca 360
ggcattgcga tagccaaaac cataaggctt gagagtgaag tgaatgcaat caaaggtgct 420
ctcaaaacaa ccaacgaggc agtatccaca ctaggaaatg gagtgcgagt cctagccact 480
gcagtaagag agctgaaaga atttgtgagc aaaaacctga ctagtgcaat taacaagaac 540
aaatgtgaca ttgctgatct gaagatggct gtcagcttca gtcaattcaa cagaagattc 600
ctaaatgttg tgcggcagtt ttcagacaat gcagggataa caccagcaat atcattagac 660
ctaatgactg atgctgagct ggccagagct gtatcataca tgccaacatc tgcaggacag 720
ataaaactaa tgttagagaa ccgtgcaatg gtaagaaga 759

<210> 55
<211> 759
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(759)
<223> human metapneumovirus, strain Quebec Isolate No C-78429

<400> 55
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gagagttatt tggaagaatc atgcagtact ataactgagg gatacctcag tgttttaaga 120
acaggctggt acattaatgt cttcacatta gaagttggtg atgttgaaaa tcttacatgt 180
actgatggac ctagcttaat caaaacagaa cttgacctaa caaaaagtgc tttaagggaa 240
ctcaaaacag tctctgctga tcagttagcg agaggaggagc aaattgaaaa tcccagacaa 300
tcaagatttg tcctaggtgc aatagctctc ggagttgcta cagcagcagc agtcacagca 360
ggcattgcaa tagccaaaac cataaggctt gagagtgagg tgaatgcaat taaaggtgct 420
ctcaaacaaa ctaatgaagc agtatccaca ttaggaaatg gtgtgcgggt cctagccact 480
gcagtgagag agctaaaaga atttgtgagc aaaaatctga ctagtgcaat caacaggaac 540
aaatgtgaca ttgctgatct gaagatggct gtcagcttca gtcaattcaa cagaagattt 600
ctcaatgttg tgcggcagtt ttcagacaat gcagggataa caccagcaat atcattggac 660
ctaatgactg atgctgaatt ggccagagct gtatcataca tgccaacatc tgcaggacag 720
ataaaactga tgttggagaa ccgtgcaatg gtaagaaga 759

<210> 56
<211> 759
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(759)
<223> human metapneumovirus, strain Quebec Isolate No C-78652

<400> 56
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gagagttatt tggaagaatc atgcagtact ataactgagg gatacctcag tgttttaaga 120
acgggctggt acactaatgt cttcacatta gaagttggtg atgttgaaaa tcttacatgt 180
actgatggac ctagcttaat caaaacagaa cttgacctaa caaaaagtgc tttaagggaa 240
ctcaaaacag tctctgctga tcagttaacg agagaggagc aaattgaaaa tcccagacaa 300
tcaagatttg tcctaggtgc aatagctctc ggagttgcta cagcagcagc agtcacagca 360
ggcattgcaa tagccaaaac cataaggctt gagagtgagg tgaatgcaat taaaggtgct 420
ctcaaacaaa ctaatgaagc agtatccaca ttaggaaatg gtgtgcgggt cctagccact 480
gcagtgagag agctaaaaga atttgtgagc aaaaatctga ctagtgcaat caacaggaac 540
aaatgtgaca ttgctgatct gaagatggct gtcagcttca gtcaattcaa cagaagattt 600
ctaaatgttg tgcggcagtt ttcagacaat gcagggataa caccagcaat atcattggac 660
ctaatgactg atgctgaatt ggccagagct gtatcataca tgccaacatc tgcaggacag 720
ataaaactga tgttggagaa ccgtgcaatg gtaagaaga 759

<210> 57
<211> 759
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(759)
<223> human metapneumovirus, strain Quebec Isolate No hMPV-118

<400> 57
atgtcttgga aaggggtgat tatcatttcg ttactcataa cacctcagca cggactaaaa 60
gaaagttatt tagaagaatc atgtagtact ataactgaag gatatctcag tgttttaaga 120
acaggttggt acaccaatgt ctttacatta gaagttggtg atgttgaaaa tcttacatgt 180
actgatggac ctagcttaat caaaacagaa cttgacctaa ccaaaagtgc tctgagagaa 240
ctcaaaacag tttctgctga tcagttaacg agagaagaac aaattgaaaa tcccagacaa 300
tcaaggtttg tcctaggtgc aatagctctt ggtgttgcca cagcagcagc agtcacagca 360
ggcattgcga tagccaaaac cataaggctt gagagtgaag tgaatgcaat caaaggtgct 420
ctcaaaacaa ccaacgaggc agtatccaca ctaggaaatg gagtgcgagt cctagccacc 480
gcagtaagag agctgaaaga atttgtgagc aaaaacctga ccagtgcaat taacaagaac 540
aaatgtgaca ttgctgatct gaagatggct gtcagcttca gtcaattcaa cagaagattc 600
ctaaatgttg tgcggcagtt ttcagacaat gcagggataa caccagcaat atcattagac 660
ctaatgactg atgctgagct ggccagagct gtatcataca tgccaacatc tgcagggcag 720
ataaaactaa tgttagagaa ccgtgcaatg gtaagaaga 759

<210> 58
<211> 759
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(759)
<223> human metapneumovirus, strain Quebec Isolate No hMPV-200

<400> 58
atgtcttgga aagtggtgat catttttcg ttactcataa cacctcagca cggactaaaa 60
gaaagttatt tagaagaatc atgtagtact ataactgaag gatatctcag tgttttaaga 120
acaggttggt acaccaatgt ctttacatta gaagttggtg atgttgaaaa tcttacatgt 180
actgatggac ctagcttaat caaaacagaa cttgacctaa ccaaaagtgc tctaagagaa 240
ctcaaaacag tttctgctga tcagttaacg agagaagaac aaattgaaaa tcccagacaa 300
tcaaggtttg tcctaggtgc aatagctctt ggtgttgcca cagcagcagc agtcacagca 360
ggcattgcga tagccaaaac cataaggctt gagagtgaag tgaatgcaat caaaggtgct 420
ctcaaaacaa ccaacgaggc agtatccaca ctaggaaatg gagtgcgagt cctagccacc 480
gcagtaagag agctgaaaga atttgtgagc aaaaacctga ccagtgcaat taacaagaac 540
aaatgtgaca ttgctgatct gaagatggct gtcagcttca gtcaattcaa cagaagattc 600
ctaaatgttg tgcggcagtt ttcagacaat gcagggataa caccagcaat atcattagac 660
ctaatgactg atgctgagct ggccagagct gtatcataca tgccaacatc tgcagggcag 720
ataaaactaa tgttagagaa ccgtgcaatg gtaagaaga 759

<210> 59
<211> 759
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(759)
<223> human metapneumovirus, strain Quebec Isolate No hMPV-176

<400> 59
atgtcttgga aagtggtgat tatcatttcg ttactcataa cacctcagca cggactaaaa 60
gaaagttatt tagaagaatc atgtagtact ataactgaag gatatctcag tgttctaaga 120
acaggttggt acaccaatgt ctttacatta gaagttggtg atgttgaaaa tcttacatgt 180
actgatggac ctagcttaat caaaacagaa cttgacctaa ccaaaagtgc tttaagagaa 240
ctcaaaacag tttctgctga tcagttagcg agagaagaac aaattgaaaa tcccagacaa 300
tcaaggtttg tcctaggtgc aatagctctc ggagttgcca cagcagcagc agtcacagca 360
ggcattgcaa tagccaaaac tataaggctt gagagtgaag tgaatgcaat caaaggtgct 420
ctcaaaacaa ccaatgaggc agtatcaaca ctaggaaatg gagtgcgggt cctagccact 480
gcagtaagag agctgaaaga atttgtgagc aaaaacctga ctagtgcgat caacaagaac 540
aaatgtgaca ttgctgattt gaagatggct gtcagcttca gtcagttcaa cagaagattc 600
ctaaatgttg tgcggcagtt ttcagacaat gcagggataa caccagcaat atcattggac 660
ctgatgactg atgctgagct ggccagagct gtatcataca tgccaacatc tgcaggacag 720
ataaaactaa tgttagagaa ccgtgcaatg gtaagaaga 759

<210> 60
<211> 711
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(711)
<223> human metapneumovirus, strain Quebec Isolate No hMPV-193

<400> 60
atggaggtga aagtggagaa cattcgaaca atagatatgc tcaaagcaag agtgaaaaat 60
cgtgtggcac gcagcaaatg ctttaaaaat gcctctttga tcctaatagg aataactaca 120
ttgagtatag ccctcaatat ctatctgatc ataaactata caatgcaaga aaacacatcc 180
gaatcagaac atcacaccag ctcatcaccc atggaatcca gcagggaaac tccaacggtc 240
cccatggact acccagacac caatccaggc tcacagtatc caactcaaca gtccacagaa 300
ggctccacac tctactttgc agcctcagca agctcaccag agacagaacc aacatcaaca 360
ccaaacacaa caagccgccc gcccttcgtc gacacacaca caacaccacc aagtgcaagc 420
agaacaaaga caagtccggc agtccacaca aaaaacaatc caaggataag cctcagaaca 480
cattccccac catgggcaat gacaaggacg gtccgcggaa ccaccactct ccgcacaagc 540
agcacaagaa aaagaccgtc tacagcatca gtccaacctg acagcagcgc aacaacccac 600
aaacacgaag aagcaagccc agtgagcccg caaacatctg caagcacagc aagaccacaa 660
aggaaggtca tggaggccag cacatcaaca acatacaatc aaactagtta a 711

<210> 61
<211> 711
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(711)
<223> human metapneumovirus, strain Quebec Isolate No hMPV-228

<400> 61
atggaggtga aagtggagaa cattcgaaca atagatatgc tcaaagcaag agtgaaaaat 60
cgtgtggcac gcagcaaatg ctttaaaaat gcctctttga tcctaatagg aataactaca 120
ttgagtatag ccctcaatat ctatctgatc ataaactata caatgcaaga aaacacatcc 180
gaatcagaac atcacaccag ctcatcaccc atggaatcca gcagggaaac tccaacggtc 240
cccatggact acccagacac caatccaggc tcacagtatc caactcaaca gtccacagaa 300
ggctccacac tctactttgc agcctcagca agctcaccag agacagaacc aacatcaaca 360
ccaaacacaa caagccgccc gcccttcgtc gacacacaca caacaccacc aagtgcaagc 420
agaacaaaga caagtccggc agtccacaca aaaaacaatc caaggataag cctcagaaca 480
cattccccac catgggcaat gacaaggacg gtccgcggaa ccaccactct ccgcacaagc 540
agcacaagaa aaagaccgtc tacagcatca gtccaacctg acagcagcgc aacaacccac 600
aaacacgaag aagcaagccc agtgagcccg caaacatctg caagcacagc aagaccacaa 660
aggaaggtca tggaggccag cacatcaaca acatacaatc aaactagtta a 711

<210> 62
<211> 711
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(711)
<223> human metapneumovirus, strain Quebec Isolate No C-77748

<400> 62
atggaggtga aagtggagaa cattcgaaca atagatatgc tcaaagcaag agtgaaaaat 60
cgtgtggcac gcagcaaatg ctttaaaaat gcctctttga tcctaatagg aataactaca 120
ttgagtatag ccctcaatat ctatctaatc ataaactata caatgcaaga aaacacatcc 180
gaatcagaac atcacaccag ctcatcaccc atggaatcca gcagggaaac tccaacagtc 240
cctatggaca actcagacac caatccaggc tcacagtatc caactcaaca gtccacagaa 300
ggctccacac tctactttgc agcctcagca agctcaccag agacagaacc aacatcaaca 360
ccagacacaa caagccgccc gcccttcgtc gacacacaca caacaccacc aagtgcaagc 420
agaacaagga caagtccggc agtccacaca aaaaacaatc caaggacaag ccccagaaca 480
cattccccac catgggcaat gacaaggacg gtccgtggaa ccaccactct ccgcacaagc 540
agcacaagaa aaagaccgtc tacagcatca gtccaacctg acagcagcgc aacaacccac 600
aaacacgaag aagcaagccc agtgagcccg caaacatctg cgagcacagc aagaccacaa 660
aggaagggca tggaggccag cacatcaaca acatacaacc aaactagtta a 711

<210> 63
<211> 711
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(711)
<223> human metapneumovirus, strain Quebec Isolate No C-78132

<400> 63
atggaggtga aagtggagaa cattcgaaca atagatatgc tcaaagcaag agtgaaaaat 60
cgtgtggcac gcagcaaatg ctttaaaaat gcctctttga tcctaatagg aataactaca 120
ttgagtatag ccctcaacat ctatctgatc ataaactata caatgcaaga aaacacatcc 180
gaatcagaac atcacaccag ctcatcaccc atggaatcca gcagggaaac tccaacggtc 240
cctatagaca actcagacac caatccaggc tcacagtatc caactcaaca gtccacagaa 300
ggctccacac tccactttgc agcctcagca agctcaccag agacagaacc aacatcaaca 360
ccagacacaa caagccgccc gcccttcgtc gacacacaca caacaccacc aagtgcaagc 420
agaacaagga caagtccggc agtccacaca aaaaacaatc taaagataag ccccagaata 480
cattccccac catgggcaat gacaaggacg gtccgtggaa ccaccactct ccgcacaagc 540
agcacaagaa aaagaccgtc tacagcatca gtccaacctg acagcagcac aacaactcac 600
aaacacgaag aagcaagccc agtgagccca caaacatctg caagcacagc aagaccacaa 660
aggaagggca tggaggccag cacatcaaca acatacaacc aaactagtta a 711

<210> 64
<211> 711
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(711)
<223> human metapneumovirus, strain Quebec Isolate No C-78406

<400> 64
atggaggtga aagtggagaa cattcgaaca atagatatgc tcaaagcaag agtgaaaaat 60
cgtgtggcac gcagcaaatg ctttaaaaat gcctctttga tcctaatagg aataactaca 120
ttgagtatag ccctcaacat ctatctgatc ataaactata caatgcaaga aaacacatcc 180
gaatcagaac atcacaccag ctcatcaccc atggaatcca gcagggaaac tccaacggtc 240
cctatagaca actcagacac caatccaggc tcacagtatc caactcaaca gtccacagaa 300
ggctccacac tccactttgc agcctcagca agctcaccag agacagaacc aacatcaaca 360
ccagacacaa caagccgccc gcccttcgtc gacacacaca caacaccacc aagtgcaagc 420
agaacaagga caagtccggc agtccacaca aaaaacaatc taaagataag ccccagaata 480
cattccccac catgggcaat gacaaggacg gtccgtggaa ccaccactct ccgcacaagc 540
agcacaagaa aaagaccgtc tacagcatca gtccaacctg acagcagcac aacaactcac 600

aaacacgaag aagcaagccc agtgagccca caaacatctg caagcacagc aagaccacaa 660
aggaagggca tggaggccag cacatcaaca acatacaacc aaactagtta a 711

<210> 65
<211> 711
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(711)
<223> human metapneumovirus, strain Quebec Isolate No C-73750

<400> 65
atggaggtga aagtggagaa cattcgaaca atagatatgc tcaaagcaag agtgaaaaat 60
cgtgtggcac gcagcaaatg ctttaaaaat gcctctttga tcctaatagg aataactaca 120
ttgagtatag ccctcaacat ctatctgatc ataaactata caatgcaaga aaacacatcc 180
gaatcagaac atcacaccag ctcatcaccc atggaatcca gcagggaaac tccaacggtc 240
cctatagaca actcagacac caatccaggc tcacagtatc caactcaaca gtccacagaa 300
ggctccacac tccactttgc agcctcagca agctcaccag agacagaacc aacatcaaca 360
ccagacacaa caagccgccc gcccttcgtc gacacacaca caacaccacc aagtgcaagc 420
agaacaagga caagtccggc agtccacaca aaaaacaatc taaagataag ccccagaaca 480
cattccccac catgggcaat gacaaggacg gtccgtggaa ccaccactct ccgcacaagc 540
agcataagaa aaagaccgtc cacagcatca gtccaacctg acagcagcac aacaacccac 600
aaacacgaag aagcaagccc agtgagccca caaacatctg caagcacagc aagaccacaa 660
aggaagggca tggaggccag cacatcaaca acacacaacc aaactagtta a 711

<210> 66
<211> 660
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(660)
<223> human metapneumovirus, strain Quebec Isolate No C-60298

<400> 66
atggaggtga aagtggagaa cattcgagca atagacatgc tcaaagcaag agtgaaaaat 60
cgtgtggcac gtagcaaatg ctttaaaaat gcttctttaa tcctcatagg aataactaca 120
ctgagtatag ctctcaatat ctatctgatc ataaactaca caatacaaaa aacctcatct 180
gaatcagaac accacaccag ctcaccaccc acagaatcca acaaggaagc ttcaacaatc 240
tccacagaca acccagacat caatccaaac tcacagcatc caactcaaca gtccacagaa 300
aaccccacac tcaaccccgc agcatcagtg agcccatcag aaacagaacc agcatcaaca 360
ccagacacaa caaaccgcct gtcctccgta gacaggtcca cagcacaacc aagtgaaagc 420
agaacaaaga caaaaccgac agtccacaca agaaacaacc caagcacagc ttccagtaca 480
caatccccac cacgggcaac aacgaaggca atccgcagag ccaccacttt ccgcatgagc 540
agcacaggaa aaagaccaac cacaacatca gtccagtccg acagcagcac cacaacccaa 600
aatcatgaag aaacaggttc agcgaaccca caggcatctg taagcacaat gcaaaactag 660

<210> 67
<211> 654
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(654)
<223> human metapneumovirus, strain Quebec Isolate No C-74783

<400> 67
atggaggtga aagtggagaa cattcgagca atagacatgc tcaaagcaag agtgaaaaat 60
cgtgtggcat gtagcaaatg ctttaaaaat gcttctttaa tcctcatagg aataactaca 120
ctgagtatag ctctcaatat ctatttgatc ataaaccaca caatacaaaa aaccacatct 180
gaatcagaac accacaccag ctcaccaccc acagaatcta acaaggaagc ttcaacaatc 240
tccacagaca acccagacat caatccaaac tcacagcatc caactcaaca gtctacagaa 300
aaccccacac tcaaccccgc agtatcaaca agcccatcag aaacagaacc aacatcaaca 360
ccagacacaa caaaccgcct gtcctccgta gacaggtcca cagcacaacc aagtgaaagc 420
agaacaaaga caaaatcgac agtccacaca agaaacaacc caagcacagc ttccagcaca 480

caatccccac cacgggcaac aacgaaggca atccgcagag ccaccacttt ccgcatgagc 540
agcacagaaa aaagaccaac cacaacacta gtccagtacg acagcagcac cacaacccaa 600
aatcatgaag aaacaggttc agcgaaccca caggcatctg caagcacaat gtaa 654

<210> 68
<211> 660
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(660)
<223> human metapneumovirus, strain Quebec Isolate No C-74549

<400> 68
atggaggtga aagtggagaa cattcgagca atagacatgc tcaaagcaag agtgaaaaat 60
cgtgtggcac gtagcaaatg cttcaaaaat gcttccccaa tcctcacagg aataaccaca 120
ctgagcatag ctctcaatat ccacctgatc ataaactaca caatacaaaa aaccacatct 180
gaatcagaac accacaccag ctcaccaccc acagaatcta acaaggaagc ttcaacaatc 240
tccacagaca acccagacat caatccaaac tcacagcatc caactcaaca gtctacagaa 300
aaccccacac tcaaccccgc agtatcaaca agcccatcag aaacagaacc aacatcaaca 360
ccagacacaa caaaccgcct gtcctccgta gacaggtcca cagcacaacc aagtgaaagc 420
agaacaaaga caaaatcgac agtccacaca agaaacaacc caagcacagc ttccagcaca 480
caatccccac cacgggcaac aacgaaggca atccgcagag ccaccacttt ccgcatgagc 540
agcacagaaa aaagaccaac cacaacatta gtccagtacg acagcagcac cacaacccaa 600
aatcatgaag aaacaggttc agcgaaccca caggcatctg taagcacaat gtaaaactag 660

<210> 69
<211> 711
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(711)
<223> human metapneumovirus, strain Quebec Isolate No C-77807

<400> 69
atggaggtga aagtggagaa cattcgaaca atagatatgc tcaaagcaag agtgaaaaat 60
cgtgtggcac gcagcaaatg ctttaaaaat gcctctttga tcctaatagg aataacgaca 120
ttgagtatag ccctcaacat ctatctgatc ataaactata caatgcaaga aaacacatcc 180
gaatcagaac atcacaccag ctcatcaccc atggaatcca gcagggaaac tccaacggtc 240
cctatagaca actcagacac caatccaggc tcacagtatc caactcaaca gtccacagaa 300
ggctccacac tccactttgc agcctcagca agctcaccag agacagaacc aacatcaaca 360
ccagacacaa caagccgccc gcccttcgtc gacacacaca caacaccacc aagtgcaagc 420
agaacaagga caagtccggc agtccacaca aaaaacaatc taaagataag ccccagaata 480
cattccccac catgggcaat gacaaggacg gtccgtggaa ccaccactct ccgcacaagc 540
agcacaagaa aaagaccgtc tacagcatca gtccaacctg acagcagcac aacaactcac 600
aaacacgaag aagcaagccc agtgagccca caaacatctg caagcacagc aagaccacaa 660
aggaagggca tggaggccag cacatcaaca acatacaacc aaactagtta a 711

<210> 70
<211> 711
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(711)
<223> human metapneumovirus, strain Quebec Isolate No C-78163

<400> 70
atggaggtga aagtggagaa cattcgaaca atagatatgc tcaaagcaag agtgaaaaat 60
cgtgtggcac gcagcaaatg ctttaaaaat gcctctttga tcctaatagg aataactaca 120
ttgagtatag ccctcaacat ctatctgatc ataaactata caatgcaaga aaacacatcc 180
gaatcagaac atcacaccag ctcatcaccc atggaatcca gcagggaaac tccaacggtc 240
cctatagaca actcagacac caatccaggc tcacagtatc caactcaaca gtccacagaa 300
ggctccacac tccactttgc agcctcagca agctcaccag agacagaacc aacatcaaca 360
ccagacacaa caagccgccc gcccttcgtc gacacacaca caacaccacc aagtgcaagc 420

agaacaagga caagtccggc agtccacaca aaaaacaatc taaagataag ccccagaata 480
cattccccac catgggcaat gacaaggacg gtccgtggaa ccaccactct ccgcacaagc 540
agcacaagaa aaagaccgtc tacagcatca gtccaacctg acagcagcac aacaactcac 600
aaacacgaag aagcaagccc agtgagccca caaacatctg caagcacagc aagaccacaa 660
aggaagggca tggaggccag cacatcaaca acatacaacc aaactagtta a 711

<210> 71
<211> 711
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(711)
<223> human metapneumovirus, strain Quebec Isolate No C-68661

<400> 71
atggaggtga aagtggagaa cattcgaaca atagatacgc tcaaagcaag agtgaaaaat 60
cgtgtggcac gcagcaaatg ctttaaaaat gcctctttga tcctaatagg aataactaca 120
ttgagtatag ccctcaatat ctatctgatc ataaactata caatgcaaga aaacacatcc 180
aaatcagaac atcacaccag ctcatcaccc atggaatcca gcagggaaac tccaacggtc 240
cctatagaca actcagacac caatccaggc tcacagtatc caactcaaca gtccacagaa 300
ggctccacac tccactttgc agcctcagca agctcaccag agacagaacc aacatcaaca 360
ccagacacaa caagccgccc gcccttcgtc gacacacaca caacaccacc aagtgcaagc 420
agaacaagga caagtccggc agtccacaca aaaaacaatc caaggataag ccccagaaca 480
cattccccac catgggcaat gacaaggacg gtccgtggaa ccaccactct ccgcacaagc 540
agcacaagaa aaagaccgtc tacagcatca gtccaacccg acagcagcgc aacaacccac 600
aaacacgaag aagcaagccc agtgagccca caaacatctg caagcacagc aagaccacaa 660
aggaagggca tggaggccag cacatcaaca acatacaacc aaactagtta a 711

<210> 72
<211> 711
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(711)
<223> human metapneumovirus, strain Quebec Isolate No C-88448

<400> 72
atggaggtga aagtggagaa cattcgaaca atagatatgc tcaaagcaag agtgaaaaat 60
cgtgtggcac gcagcaaatg ctttaaaaat gcctctttga tcctaatagg aataactaca 120
ttgagtatag ccctcaatat ctatctaatc ataaactata caatgcaaga aaacacatcc 180
gaatcagaac atcacaccag ctcatcaccc atggaatcca gcagggaaac tccaacagtc 240
cctatggaca actcagacac caatccaggc tcacagtatc taactcaaca gtccacagaa 300
ggctccacac tctactttgc agcctcagca agctcaccag agacagaacc aacatcaaca 360
ccagacacaa caagccgccc gcccttcgtc gacacacaca caacaccacc aagtgcaagc 420
agaacaagga caagtccggc agtccacaca aaaaacaatc caaggacaag ccccagaaca 480
cattccccac catgggcaat gacaaggacg gtccgtggaa ccaccactct ccgcacaagc 540
agcacaagaa aaagaccgtc cacagcatca gtccaacctg acagcagcgc aacaacccac 600
aaacacgaag aagcaagccc agtgagcccg caaacatctg cgagcacagc aagaccacaa 660
aggaagggca tggaggccag cacatcaaca acatacaacc aaactagtta a 711

<210> 73
<211> 711
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(711)
<223> human metapneumovirus, strain Quebec Isolate No C-88470

<400> 73
atggaggtga aagtggagaa cattcgaaca atagatatgc tcaaagcaag agtgaaaaat 60
cgtgtggcac gcagcaaatg ctttaaaaat gcctctttga tcctaatagg aataactaca 120
ttgagtatag ccctcaatat ctatctgatc ataaactata caatgcaaga aaacacatcc 180
gaatcagaac atcacaccag ctcatcaccc atggaatcca gcagggaaac tccaacggtc 240

ccatggact acccagacac caatccaggc tcacagcatc caactcaaca gtccacagaa 300
ggctccacac tctactttgc agcctcagca agctcaccag agacagaacc aacatcaaca 360
ccaaacacaa caagccgccc gcccttcgtc gacacacaca caacaccacc aagtgcaagc 420
agaacaaaga caagtccggc agtccacaca aaaaacaatc caaggataag cctcagaaca 480
cattccccac catgggcaat gacaaggacg gtccgcggaa ccaccactct ccgcacaagc 540
agcacaagaa aaagaccgtc tacagcatca gtccaacctg acagcagcgc aacaacccac 600
aaacacgaag aagcaagccc agtgagcccg caaacatctg cgagcacagc aagaccacaa 660
aggaaggtca tggaggccag cacatcaaca acatacaacc aaactagtta a 711

<210> 74
<211> 711
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(711)
<223> human metapneumovirus, strain Quebec Isolate No C-86316

<400> 74
atggaggtga aagtggagaa cattcgaaca atagatatgc tcaaagcaag agtgaaaaat 60
cgtgtggcac gcagcaaatg ctttaaaaat gcctctttga tcctaatagg aataactaca 120
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<211> 450
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<210> 78
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<210> 79
<211> 450
<212> DNA
<213> human metapneumovirus

<220>

<221> gene
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<211> 500
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
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<223> human metapneumovirus, strain Quebec Isolate No hMPV-196

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tgtatcgtca agagcaacaa aagcaaaaga gtgagcttct tcacatacaa tcaactgtta 480
acatggaaag atgtgatgtt 500

<210> 81
<211> 450
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(450)
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<210> 82
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<212> DNA
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<220>
<221> gene
<222> (1)...(450)
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<400> 82
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<210> 83
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<220>
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<210> 84
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<220>
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<223> human metapneumovirus, strain Quebec Isolate No C-78123

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<210> 85
<211> 450
<212> DNA
<213> human metapneumovirus

<220>
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<222> (1)...(450)
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<400> 85
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<210> 86

<211> 400
<212> DNA
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<220>
<221> gene
<222> (1)...(400)
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<400> 87
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<223> human metapneumovirus, strain Quebec Isolate No hMPV-Q86

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aactggttta gtaattggta caatctcaat aagttaattt tagaattcag gagagaggaa 300
gtaataagaa ccggttcaat cttgtgcagg tcattaggta aattagtttt tattgtatca 360
tcatatggat gtatcgtcaa gagcaacaaa agcaaaagag tgagcttctt cacatacaat 420
caactgttaa catggaaaga tgtgatgtta 450

<210> 89
<211> 450
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(450)
<223> human metapneumovirus, strain Quebec Isolate No hMPV-Q97

<400> 89
aaaatgaaaa tatcagatta caaggtagta gagccagtaa acatgcaaca tgaaataatg 60
aagaatgtac acagttgtga gctcacacta ttgaaacagt ttttaactag gagtaaaaac 120
attagcactc tcaaattaaa tatgatatgt gattggctgc aattaaagtc tacatcagat 180
gatacctcaa tcctaagttt catagatgta gaatttatac ctagttgggt aagcaactgg 240
tttagtaatt ggtacaatct caataagtta attttggaat tcagaagaga agaagtaata 300
agaaccggtt caatcttatg caggtcattg ggtaaattag tttttattgt atcatcatat 360
ggatgtatcg tcaagagcaa caaaagcaaa agagtgagct tcttcacata caatcaactg 420
ttaacatgga aagatgtgat gttaagtaga 450

<210> 90
<211> 537
<212> DNA
<213> human metapneumovirus

<220>
<221> gene
<222> (1)...(537)
<223> human metapneumovirus, strain Can 97-83

<400> 90
agaatcctgt tattgagcat gtgagactca aaaatggcgg tcattctaaa atgaaaatat 60
cagattacaa ggtagtagag ccagtaaaca tgcaacatga aataatgaag aatgtacaca 120
gttgtgagct cacactattg aaacagtttt taacaaggag taaaaacatt agcactctca 180
aattgaatat gatatgtgat tggctgcaat taaagtctac atcagatgat acctcaatcc 240
taagtttcat agatgtagaa tttataccta gttgggtaag caactggttt agtaattggt 300
acaatctcaa taagttaatt ttggaattca gaagagagga agtaataaga accggttcaa 360
tcttatgcag gtcattgggt aaattagttt ttattgtatc atcatatgga tgtatcgtca 420
agagcaacaa aagcaaaaga gtgagcttct tcacatacaa tcaactgtta acatggaaag 480
atgtgatgtt aagtagattt aatgcgaatt tttgtatatg ggtaagcaac agttctg 537

<210> 91
<211> 20
<212> DNA
<213> human metapneumovirus

<220>
<223> primer no 55

<400> 91
atgggacaag tgaaaatgtc 20

<210> 92
<211> 19
<212> DNA
<213> human metapneumovirus

<220>
<223> primer no 56

<400> 92
gagtctcagt acacaataa 19

<210> 93
<211> 18
<212> DNA
<213> human metapneumovirus

<220>
<223> primer no 57

<400> 93
gtcactgcag ttgttgta 18

<210> 94
<211> 19
<212> DNA
<213> human metapneumovirus

<220>
<223> primer no 58

<400> 94
gcatttccga gaacaacac 19

<210> 95
<211> 19
<212> DNA
<213> human metapneumovirus

<220>
<223> primer no 18 multiplex assay

<400> 95
gagtctcagt acacaataa 19

<210> 96
<211> 19
<212> DNA
<213> human metapneumovirus

<220>
<223> primer no 19 multiplex assay

<400> 96
gcatttccga gaacaacac 19

<210> 97
<211> 18
<212> DNA
<213> human metapneumovirus

<220>
<223> primer no 59

<400> 97
atgtcttgga aagtggtg 18

<210> 98
<211> 17
<212> DNA
<213> human metapneumovirus

<220>
<223> primer no 60

<400> 98
tcttcttacc attgcac 17

<210> 99
<211> 20
<212> DNA
<213> human metapneumovirus

<220>
<223> primer no 61

<400> 99
ctaattatgt ggtatgaagc 20

<210> 100
<211> 18
<212> DNA
<213> human metapneumovirus

<220>
<223> primer no 62

<400> 100
atggagtcct acctagta 18

<210> 101
<211> 19

<212> DNA
<213> human metapneumovirus

<220>
<223> primer no 63

<400> 101
aaggtgcttg gttgttatc 19

<210> 102
<211> 19
<212> DNA
<213> human metapneumovirus

<220>
<223> primer no 64

<400> 102
atgtcattcc ctgaaggaa 19

<210> 103
<211> 19
<212> DNA
<213> human metapneumovirus

<220>
<223> primer no 65

<400> 103
ttaactggta aatctcatc 19

<210> 104
<211> 19
<212> DNA
<213> human metapneumovirus

<220>
<223> primer no 66

<400> 104
catgggacaa gtagttatg 19

<210> 105
<211> 19
<212> DNA
<213> human metapneumovirus

<220>
<223> primer no 67

<400> 105
atggaggtga aagtggaga 19

<210> 106
<211> 19
<212> DNA
<213> human metapneumovirus

<220>
<223> primer no 68

<400> 106
ttaactagtt tggttgtat 19

<210> 107
<211> 19
<212> DNA
<213> human metapneumovirus

<220>
<223> primer no 69

<400> 107
:ttagagtta ttttgtatt 19

<210> 108
<211> 24
<212> DNA
<213> human metapneumovirus

<220>
<223> primer no 70

<400> 108
ggacaagtag ttatggaggt gaaa 24

<210> 109
<211> 28
<212> DNA
<213> human metapneumovirus

<220>
<223> primer no 71

<400> 109
ctcctttaag atatgagtca ggmagrta 28

<210> 110
<211> 22
<212> DNA
<213> human metapneumovirus

<220>
<223> primer no 20 multiplex assay

<400> 110
gttgccatag agaatcctgt ta 22

<210> 111
<211> 22
<212> DNA
<213> human metapneumovirus

<220>
<223> primer no 21 multiplex assay

<400> 111
cattcagact gttgcttacc ca 22

<210> 112
<211> 22
<212> DNA
<213> human metapneumovirus

<220>
<223> primer no 72

<400> 112
gttgccatag agaatcctgt ta 22

<210> 113
<211> 22
<212> DNA
<213> human metapneumovirus

<220>
<223> primer no 73

<400> 113
cattcagact gttgcttacc ca 22

<210> 114
<211> 27
<212> DNA

<213> human Metapneumovirus

<220>
<223> human Metapneumovirus
primer A1

<400> 114
ccaattacta aaccaattgc ttaccca 27

<210> 115
<211> 21
<212> DNA
<213> human metapneumovirus

<220>
<223> probe no 74

<400> 115
gcaagcttat ggggccggtc a 21

<210> 116
<211> 25
<212> DNA
<213> human metapneumovirus

<220>
<223> probe no 75

<400> 116
acaatgctaa ggtgggggt cattg 25

<210> 117
<211> 23
<212> DNA
<213> human metapneumovirus

<220>
<223> probe no 76

<400> 117
gttctgagga caggttggta cac 23

<210> 118
<211> 23
<212> DNA
<213> human metapneumovirus

<220>
<223> probe no 77

<400> 118
aatgttttta cactggaggt agg 23

<210> 119
<211> 22
<212> DNA
<213> human metapneumovirus

<220>
<223> probe no 78

<400> 119
gttacaatac cagcattcat ca 22

<210> 120
<211> 25
<212> DNA
<213> human metapneumovirus

<220>
<223> probe no 79

<400> 120
atcagtttca atcaaagaga gtgag 25

<210> 121
<211> 25
<212> DNA
<213> human metapneumovirus

<220>
<223> probe no 80

<400> 121
taaagcaagc aatcaaagtc atgga 25

<210> 122
<211> 25
<212> DNA
<213> human metapneumovirus

<220>
<223> probe no 81

<400> 122
cccattgaag aagaagagtc taccg 25

<210> 123
<211> 20
<212> DNA
<213> human metapneumovirus

<220>
<223> probe no 82

<400> 123
actcaagccc acagcatcca 20

<210> 124
<211> 23
<212> DNA
<213> human metapneumovirus

<220>
<223> probe no 83

<400> 124
ctcaacagtc cacagaaggc tcc 23

<210> 125
<211> 26
<212> DNA
<213> human metapneumovirus

<220>
<223> probe no 84

<400> 125
attggtttag taattggtac aatctc 26

<210> 126
<211> 26
<212> DNA
<213> human metapneumovirus

<220>
<223> probe no 85

<400> 126
acaagttgat tctggaattc aggaaa 26

<210> 127
<211> 25
<212> DNA

<213> human Metapneumovirus

<220>
<223> human Metapneumovirus
probe A2

<400> 127
cgagcatgtt agactcaaaa atgca 25

<210> 128
<211> 23
<212> DNA
<213> human metapneumovirus

<220>
<223> probe no 33 multiplex assay

<400> 128
atgtatccgt ccaagctgag tta 23

<210> 129
<211> 26
<212> DNA
<213> human metapneumovirus

<220>
<223> probe no 34 multiplex assay

<400> 129
attggtttag taattggtac aatctc 26

<210> 130
<211> 1027
<212> DNA
<213> human Influenza A virus

<220>
<221> gene
<222> (1)...(1027)
<223> human Influenza A virus
strain A/Hong Kong/1180/99(H3N2)
accession number af386771

<400> 130
agcaaaagca ggtagatatt gaaagatgag ccttctaccc gaggtcgaaa cgtatgttct 60
ctctatcgtt ccatcaggcc ccctcaaagc cgaaatcgcg cagagacttg aagatgtctt 120
tgctgggaaa aacacagatc ttgaggctct catggaatgg ctaaagacaa gaccaatcct 180
gtcacctctg actaagggga ttttagggtt tgtgttcacg ctcaccgtgc ccagtgagcg 240
aggactgcag cgtagacgct ttgtccaaaa tgccctcaat gggaatgggg atccaaataa 300
catggacaaa gcagttaaac tgtatagaaa acttaagagg gagataacat tccatggggc 360
caaagaaata gcactcagtt attctgctgg tgcacttgcc agttgcatgg gcctcatata 420
caataggatg ggggctgtaa ccactgaagt ggcatttggc ctggtatgtg caacatgtga 480
acagattgct gactcccagc acaggtctca taggcaaatg gtggcaacaa ccaatccatt 540
aataagacat gagaacagaa tggttttggc cagcactaca gctaaggcta tggagcaaat 600
ggctggatca agtgagcagg cagcggaggc catggagatt gctagtcagg ccaggcaaat 660
ggtgcaggca atgagagccg ttgggactca tcctagctcc agtactggtc taagagatga 720
tcttcttgaa aatttgcaga cctatcagaa acggatgggg gtgcagatgc aacgattcaa 780
gtgacccgct tgttgttgcc gcgagtatca ttgggatctt gcacttgata ttgtggattc 840
ttgatcgtct ttttttcaaa tgcatctatc gactcttcaa acacggcctt aaaagagggc 900
cttctacgga aggagtacct gagtctatga gggaagaata tcgaaaggaa cagcagaatg 960
ctttggatgc tgaccacagt cattttgtca gcataaagtt ggagtaaaaa actaccttgt 1020
ttctact 1027

<210> 131
<211> 987
<212> DNA
<213> human Influenza A virus

<220>
<221> gene
<222> (1)...(987)
<223> human Influenza A virus

strain A/Charlottesville/31/95 (H1N1)
accession number af398876

<400> 131
atgagtcttc taaccgaggt cgaaacgtac gttctctcta tcgtcccgtc aggcccctc 60
aaagccgaga tcgcacagag acttgaagat gtctttgctg gaaagaacac cgatcttgag 120
gctctcatgg agtggctaaa gacaagaccg atcctgtcac ctctgactaa ggggatttta 180
ggatttgtgt tcacgctcac cgtgcccagt gagcgaggac tgcagcgtag acgctttgtc 240
caaaatgccc ttaatgggaa tggggatcca ataaacatgg acagagcagt taaactgtat 300
agaaagctta agagggagat aacattccat ggggccaaag aaatagcact cagttattct 360
gctggtgcac ttgccagttg tatgggcctc atatacaaca ggatgggggc tgtgaccacc 420
gaatcagcat ttggcctgat atgcgcaacc tgtgaacaga ttgctgactc ccagcataag 480
tctcataggc aaatggtaac aacaaccaat ccattaataa gacatgagaa cagaatggtt 540
ctggccagca ctacagctaa agctatggag caaatggctg gatcgagtga acaagcagct 600
gaggccatgg aggttgctag tcaggccagg caaatggtgc aggcaatgag agccattggg 660
actcatccta gctctagcac tggtctgaaa aatgatcttc ttgaaaattt gcaggcctat 720
cagaaacgaa tgggggtgca gatgcaacga ttcaagtgat cctcttgttg ttgccgcaag 780
tatcattggg attgtgcacc tgatattgtg gattattgat cgccttttct tcaaatgcat 840
ttatcgtatc tttaaacacg gtctgaaaag agggcgttct acggaaggag taccagagtc 900
tatgagggaa gaatatcgag aggaacagca gaatgctgtg gatgctgacg aaggtcattt 960
tgtcagcata gagctggagt aaaaaac 987

<210> 132
<211> 1076
<212> DNA
<213> human Influenza B virus

<220>
<221> gene
<222> (1)...(1076)
<223> human Influenza B virus
strain B/Nagoya/20/99

<400> 132
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aaagcagaac tagcagaaaa attacactgt tggttcggtg ggaaagaatt tgacctagac 120
tctgccttgg aatggataaa aaacaaaaga tgcttaactg atatacaaaa agcactaatt 180
ggtgcctcta tctgcttttt aaaacccaaa gaccaagaaa gaaaaagaag attcatcaca 240
gagccctat caggaatggg aacaacagca acaaaaaaga aaggcctgat tctagctgag 300
agaaaaatga gaagatgtgt gagctttcat gaagcatttg aaatagcaga aggccatgaa 360
agctcagcgc tactatattg tctcatggtc atgtacctga accctggaaa ttattcaatg 420
caagtaaaac taggaacgct ctgtgctttg tgcgagaaac aagcatcaca ttcacacagg 480
gctcatagca gagcagcgag atcttcagtg cctggagtga ggcgagaaat gcaaatggtc 540
tcagctatga acacagcaaa aacaatgaat ggaatgggaa agggagaaga cgtccaaaaa 600
ctggcagaag aactgcaaag caacattgga gtgttgagat ctcttggagc aagtcaaaag 660
aatggggaag gaattgcaaa ggatgtaatg gaagtgctaa agcagagctc tatgggaaat 720
tcagctcttg tgaagaaata cctataatgc ttgaaccatt tcagattctt tcaatttgtt 780
cttttatctt atcagctctc catttcatgg cttggacaat agggcatttg agtcaaataa 840
aaagaggagt aaacatgaaa atacgaataa aaggtccaaa taaagagaca ataaacagag 900
aagtatcaat tttgagacac aattaccaaa aagaaatcca ggccaaagaa acaatgaagg 960
aagtgctctc tgacaacata gaagtattga gtgaccacat agtgattgag gggctttctg 1020
ctgaagagat aataaaaatg ggcaaaacag ttttggaggt agaaaaattg cattaa 1076

<210> 133
<211> 1725
<212> DNA
<213> human Respiratory Syncitial Virus

<220>
<221> gene
<222> (1)...(1725)
<223> human Respiratory Syncitial Virus
strain B1
accession number af013254

<400> 133
atggagctgc tgatccacag gttaagtgca atcttcctaa ctcttgctat taatgcattg 60
tacctcacct caagtcagaa cataactgag gagttttacc aatcgacatg tagtgcagtt 120
agcagaggtt attttagtgc tttaagaaca ggttggtata ccagtgtcat aacaatagaa 180
ttaagtaata taaaagaaac caaatgcaat ggaactgaca ctaaagtaaa acttataaaa 240
caagaattag ataagtataa gaatgcagtg acagaattac agctacttat gcaaaacaca 300
ccagctgcca acaaccgggc cagaagagaa gcaccacagt atatgaacta tacaatcaat 360

accactaaaa acctaaatgt atcaataagc aagaagagga aacgaagatt tctgggcttc 420
ttgttaggtg taggatctgc aatagcaagt ggtatagctg tatccaaagt tctacacctt 480
gaaggagaag tgaacaagat caaaaatgct ttgttatcta caaacaaagc tgtagtcagt 540
ctatcaaatg gggtcagtgt tttaaccagc aaagtgttag atctcaagaa ttacataaat 600
aaccaattat tacccatagt aaatcaacag agctgtcgca tctccaacat tgaaacagtt 660
atagaattcc agcagaagaa cagcagattg ttggaaatca acagagaatt cagtgtcaat 720
gcaggtgtaa caacaccttt aagcacttac atgttaacaa acagtgagtt actatcattg 780
atcaatgata tgcctataac aaatgatcag aaaaaattaa tgtcaagcaa tgttcagata 840
gtaaggcaac aaagttattc tatcatgtct ataataaagg aagaagtcct tgcatatgtt 900
gtacagctac ctatctatgg tgtaatagat acaccttgct ggaaattaca cacatcacct 960
ctatgcacca ccaacatcaa agaaggatca aatatttgtt taacaaggac tgatagagga 1020
tggtattgtg ataatgcagg atcagtatcc ttctttccac aggctgacac ttgtaaagta 1080
cagtccaatc gagtattttg tgacactatg aacagtttga cattaccaag tgaagtcagc 1140
ctttgtaaca ctgacatatt caattccaag tatgactgca aaattatgac atcaaaaaca 1200
gacataagca gctcagtaat tacttctctt ggagctatag tgtcatgcta tggtaaaact 1260
aaatgcactg catccaacaa aaatcgtggg attataaaga cattttctaa tggttgtgac 1320
tatgtgtcaa acaaaggagt agatactgtg tcagtgggca acactttata ctatgtaaac 1380
aagctggaag gcaagaacct ttatgtaaaa ggggaaccta taataaatta ctatgaccct 1440
ctagtgtttc cttctgatga gtttgatgca tcaatatctc aagtcaatga aaaaatcaat 1500
caaagtttag cttttattcg tagatctgat gaattactac ataatgtaaa tactggcaaa 1560
tctactacaa atattatgat aactacaatt attatagtaa tcattgtagt attgttatca 1620
ttaatagcta ttggtttgct gttgtattgc aaagccaaaa acacaccagt tacactaagc 1680
aaagaccaac taagtggaat caataatatt gcattcagca aatag 1725

<210> 134
<211> 1575
<212> DNA
<213> human Parainfluenza 1 virus

<220>
<221> gene
<222> (1)...(1575)
<223> human Parainfluenza 1 virus strain
Washington/1964
accession number nc003461

<400> 134
atggccgggc tactaagtac ttttgacaca tttagttcca ggagaagtga gagcatcaat 60
aagtctggcg gaggagcaat tatacctggt caaagaagta ccgtttctgt cttcacatta 120
ggcccgagtg tgacagatga tgcagataaa ttattaatag caaccacttt cttagcccac 180
tcattggaca cagataaaca acactctcaa agaggaggat ttttagtatc actccttgca 240
atggcctaca gtagtccgga gttatatctc actacaaacg gtgtcaatgc tgatgtcaag 300
tatgtgatat acaatatagа gagagatcct aaaagaacaa aaacagatgg gttcattgtc 360
aaaacaagag acatggagta tgaaagaaca acagagtggt tgtttggacc tatgatcaac 420
aagaacccat tgttccaagg gcaaagagag aatgcggatc tagaggcatt gcttcagaca 480
tatggatatc ctgcatgtct cggagctata atagttcaag tttggatagt gttggttaaa 540
gccataacaa gtagtgctgg tctaagaaaa ggattcttca atagattaga agcattcaga 600
caggatggaa ccgttaaaag tgccctggtc ttcacaggag acacagttga aggtattggt 660
gcagtgatga ggtcacaaca aagcttagta tctcttatgg tagaaactct ggtgactatg 720
aacacatcca ggtcagattt aactacatta gagaagaaca ttcagattgt aggaaattac 780
ataagagatg caggattagc atctttcatg aacaccatca agtatggtgt agaaacgaag 840
atggccgccc tgacactatc aaatctgaga ccagatataa acaaattgag aagccttgtt 900
gatatctatc tatcaaaggg agcccgagcc ccttttatat gtatactcag agacccagtt 960
catggagact ttgccccctgg aaactatcca gcactgtgga gctacgcaat gggcgttgct 1020
gtggtacaaa acaaagctat gcaacagtat gtaactggaa gaacatattt ggacatggaa 1080
atgttcctac ttggacaagc tgtagctaaa gatgctgatt ccaaaatcag cagtgctctg 1140
gaggaagaac taggtgtgac agatacagca aaagagagac taagacacca tctgacaaac 1200
ctttcaggag gggatggtgc gtaccacaag cctacaggtg gtggagctat agaagtggca 1260
attgatcata cagacataac atttggagtc gaggacactg ctgatcggga caacaagaac 1320
tggacaaatg acagcaatga aagatggatg aatcactcga tcagcaacca cacaatcacg 1380
attcgtggtg cagaagaact tgaagaagag acaaatgatg aagacatcac tgatatagaa 1440
aacaagattg cacgaaggct ggccgacaga aaacagagac taagccaggc aaacaataaa 1500
cgagacacca gcagtgatgc tgactatgag aatgatgatg atgctacagc ggctgcaggg 1560
ataggaggaa tttaa 1575

<210> 135
<211> 1548
<212> DNA
<213> human Parainfluenza 3 virus

<220>
<221> gene

<222> (1)...(1548)
<223> human Parainfluenza 3 virus
strain GP
accession number ab012132

<400> 135
atgttgagcc tatttgatac atttaatgca cgtaggcaag aaaacataac aaaatcagcc 60
ggtggagcta tcattcctgg acagaaaaat actgtctcta tatttgctct tggaccgaca 120
ataactgatg ataatgagaa aatgacatta gctcttcttt ttttatctca ttcactggat 180
aatgagaaac aacatgcaca aagggcaggg ttcctggtgt ccttattatc aatggcttat 240
gccaatccag agctctacct aacaacaaat ggaagtaatg cagatgtcaa gtatgtcata 300
tacatgatcg agaaagatct aaaacggcaa aagtatggag gatttgtggt taagacgaga 360
gagatggtat atgacaagac aactgattgg atatttggaa gtgacctgga ttgtgatcag 420
gaaactatgt tgcagaacgg aagaaacaat tcaacaattg aagaccttgt ccacacattt 480
gggtatccat catgtttagg agctcttata atacagatct ggatagttct ggtcaaagct 540
atcactagta tctcagggtt aagaaaaggc tttttcaccc gattggaagc tttcagacaa 600
gatggaacag tgcaggcagg gctggtattg agcggtgaca cagtggatca gattgggtca 660
attatgcggt ctcaacagag cttggtaact cttatggttg aaacattaat aacaatgaat 720
actagcagaa atgacctcac aaccatagaa aagaatatac aaattgttgg taactatata 780
agagatgcag gtctcgcttc attcttcaat acaatcagat atggaattga gactagaatg 840
gcagctttga ctctatccac tctcagacca gacatcaata gattaaaagc tttgatggaa 900
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ggtgagttcg caccaggcaa ctatcctgcc atatggagct atgcaatggg agtggcagtt 1020
gtacaaaata gagccatgca acagtatgtg acgggaagat catatctgga tattgatatg 1080
ttccagctag gacaagcagt agcacgtgat gctgaagctc agatgagctc aacactagaa 1140
gatgaacttg gagtgacaca cgaagctaaa gaaagcttga agagacatat aaggaacata 1200
aatagttcag agacatcttt ccacaaacca acaggtggat cagccataga gatggcaata 1260
gatgaagagc cagaacaatt cgaacataga tcagatcaag aacgagatgg agaacctcaa 1320
tcatctataa ttcaatatgc ttgggcagaa ggaaacagaa gcgatgatcg gactgagcaa 1380
gatacagaat ctgacaatat caagactgaa caacaaaaca tcagagacag aacaatcaag 1440
agactcaacg aaaagaagaa acaaggcagt caaccaccta ccaatcccac aaacagaacg 1500
aatcaggacg aaatagatga tctgtttaat gcatttggaa gcaactaa 1548

<210> 136
<211> 2910
<212> DNA
<213> human Adenovirus

<220>
<221> gene
<222> (1)...(2910)
<223> human Adenovirus
strain 105
accession number aj293903

<400> 136
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ctgaataaca agtttagaaa ccccacggtg gcgcctacgc acgacgtgac cacagaccgg 180
tcccagcgtt tgacgctgcg gttcatccct gtggatcgcg aggataccgc gtactcgtac 240
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tacaacgctc tagctcccaa gggcgctccc aactcctgtg agtgggaaca aaccgaagat 420
agcggccggg cggttgccga ggatgaagaa gaagaggaag atgaagatga agaaggaaa 480
gaggaagagc aaaacgctcg agatcaggct actaagaaaa cacatgttta tgcccaggct 540
cctttgtctg gagaaacaat tacaaaaagc gggctacaaa taggatcaga caatgcagaa 600
acacaagcta aacctgtata tgcagatcct tcctatcaac cagaacctca aattggcgaa 660
tctcagtgga acgaagctga tgctaatgca gcaggaggga gagtgcttaa aaaaacaact 720
cccatgaaac catgctatgg atcttatgcc aggcctacaa atcctttgg tggccaatcc 780
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aatactacct ctttgaacga ccggcaaggc aatgccacta aaccaaaagt ggtgttgtac 900
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gaaacttttg caacacgtaa tgaaatagga gtgggtaaca actttgccat ggaaattaac 1440
ctaaatgcca acctatggag aaatttcctt tactccaata ttgcactata cctgccagac 1500
aagctaaaat acaaccccac caacgtggaa atatctgaca accccaacac ctacgactac 1560

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cgttaccgct ccatgttgtt gggaaacgga cgctacgtgc cttttcacat tcaggtgccc 1740
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gcgctcacag acctgggcca aaaccttctc tacgcaaact ccgcccacgc gctagacatg 2760
acctttgagg tggatcccat ggacgagccc accttcttt atgttttgtt tgaagtcttt 2820
gacgtggtcc gtgtgcacca gccgcaccgc ggcgtcatcg agaccgtgta cctgcgcacg 2880
ccctttctcgg ccggcaacgc cacaacataa 2910

<210> 137
<211> 810
<212> DNA
<213> human Enterovirus

<220>
<221> gene
<222> (1)...(810)
<223> human Enterovirus
strain Coxakievirus B1
accession number m16560

<400> 137
cggcccctga atgcggctaa tcctaactgc ggagcacata ccctcaaacc aggggcagt 60
gtgtcgtaac gggcaactct gcagcggaac cgactacttt gggtgtccgt gtttcatttt 120
attcctatac tggctgctta tggtgacaat tgacaggttg ttaccatata gttattggat 180
tggccatccg gtgactaaca gagcaattat atatctcttt gttgggttta taccacttag 240
cttgaaagag gttaaaacac tacatctcat cattaaacta aatacaacaa aatgggggct 300
caagtatcaa cacaaaagac tggagcacat gagaccgggc tgaatgcaag cgggaattcc 360
atcatccatt acacaaacat aaactactat aaagatgctg catccaactc agccaacaga 420
caggatttca ctcaagaccc aggtaaattt actgagccag tgaaggacat tatgatcaaa 480
tcgatgccag cattgaactc ccctctgcc gaagagtgtg gttatagtga cagggttcga 540
tcaataaccc tcgggaactc cactatcaca acacaggagt gtgctaatgt tgtggttggg 600
tatggtgtgt ggccagaata tttgaaggac aatgaagcca caggcgagga ccaaccaaca 660
caacccgacg tggccacatg tcggttctac actttagagt ccgtgcagtg gatgaagaat 720
tcagcaggtt ggtggtggaa gttaccagat gcactttcac aaatggggtt gtttggacaa 780
aatatgcagt accactactt agggagaaca 810

<210> 138
<211> 681
<212> DNA
<213> human Rhinovirus

<220>
<221> gene
<222> (1)...(681)
<223> human Rhinovirus
strain 14
accession number k02121

<400> 138
taaccctaga gccttatgcc acgatccagt ggttgtaagg tcgtaatgag caattccggg 60
acgggaccga ctactttggg tgtccgtgtt tctcattttt cttcatattg tcttatggtc 120
acagcatata tatacatata ctgtgatcat gggcgctcag gtttctacac agaaaagtgg 180
atctcacgaa aatcaaaaca ttttgaccaa tggatcaaat cagactttca cagttataaa 240
ttactataag gatgcagcaa gtacatcatc agctggtcaa tcactgtcaa tggacccatc 300
taagtttaca gaaccagtta aagatctcat gcttaagggt gcaccagcat tgaattcacc 360
caatgttgag gcctgtggtt atagtgatag agtacaacaa atcacactcg ggaattcaac 420

aataacaaca caagaagcag ccaacgctgt tgtgtgttat gctgaatggc cagagtacct 480
tccagatgtg gacgctagtg atgtcaataa aacttcaaaa ccagacactt ctgtctgtag 540
gttttacaca ttggatagta agacatggac aacaggttct aaaggctggt gctggaaatt 600
accagatgca ctcaaggata tgggtgtgtt cgggcaaaac atgtttttcc actcactagg 660
aagatcaggt tacacagtac a 681

<210> 139
<211> 21
<212> DNA
<213> human Influenza A virus

<220>
<223> primer no 12 multiplex assay

<400> 139
cttctaaccg aggtcgaaac g 21

<210> 140
<211> 24
<212> DNA
<213> human Influenza A virus

<220>
<223> primer no 13 multiplex assay

<400> 140
agggcatttt ggacaaagcg tcta 24

<210> 141
<211> 20
<212> DNA
<213> human Influenza B virus

<220>
<223> primer no 14 multiplex assay

<400> 141
ttacactgtt ggttcggtgg 20

<210> 142
<211> 20
<212> DNA
<213> human Influenza B virus

<220>
<223> primer no 15 multiplex assay

<400> 142
gccagttttt ggacgtcttc 20

<210> 143
<211> 23
<212> DNA
<213> human Respiratory Syncytial Virus

<220>
<223> primer no 16 multiplex assay

<400> 143
aacagtttaa cattaccaag tga 23

<210> 144
<211> 23
<212> DNA
<213> human Respiratory Syncytial Virus

<220>
<223> primer no 17 multiplex assay

<400> 144
tcattgactt gagatattga tgc 23

<210> 145
<211> 26
<212> DNA
<213> human Parainfluenza 1 virus

<220>
<223> primer no 22 multiplex assay

<400> 145
tctggcggag gagcaattat acctgg 26

<210> 146
<211> 26
<212> DNA
<213> human Parainfluenza 1 virus

<220>
<223> primer no 23 multiplex assay

<400> 146
atctgcatca tctgtcacac tcgggc 26

<210> 147
<211> 22
<212> DNA
<213> human Parainfluenza 1 virus

<220>
<223> human Parainfluenza 1 virus
primer P1

<400> 147
gtatggtgta gaaacgaaga tg 22

<210> 148
<211> 22
<212> DNA
<213> human Parainfluenza 1 virus

<220>
<223> human Parainfluenza 1 virus
primer P2

<400> 148
ttgtaccaca gcaacaccca tt 22

<210> 149
<211> 26
<212> DNA
<213> human Parainfluenza 3 virus

<220>
<223> primer no 24 multiplex assay

<400> 149
aacaggaaac tatgctgcag aacggc 26

<210> 150
<211> 26
<212> DNA
<213> human Parainfluenza 3 virus

<220>
<223> primer no 25 multiplex assay

<400> 150
gatccactgt gtcaccgctc aatacc 26

<210> 151
<211> 25
<212> DNA
<213> human Parainfluenza virus

<220>
<223> humna Parainfluenza virus
primer P3

<400> 151
tcttgcttca ttcttcaata caatc 25

<210> 152
<211> 23
<212> DNA
<213> human Parainfluenza 3 virus

<220>
<223> human Parainfluenza 3 virus
primer P4

<400> 152
aattccatca gagcttttaa tct 23

<210> 153
<211> 18
<212> DNA
<213> human Adenovirus

<220>
<223> primer human Adenovirus P5

<400> 153
actccctcga tgatgccg 18

<210> 154
<211> 26
<212> DNA
<213> human Adenovirus

<220>
<223> primer no 26 multiplex assay

<400> 154
cctacgcacg atgtgaccac agaccg 26

<210> 155
<211> 26
<212> DNA
<213> human Adenovirus

<220>
<223> primer no 27 multiplex assay

<400> 155
gtgttgtagg cagtgccgga gtaggg 26

<210> 156
<211> 21
<212> DNA
<213> human Sars Coronavirus

<220>
<223> primer SARS no P5

<400> 156
ctaacatgct taggataatg g 21

<210> 157
<211> 21
<212> DNA
<213> human Sars Coronavirus

<220>
<223> human Sars Coronavirus
primer P6

<400> 157
caggtaagcg taaaactcat c 21

<210> 158
<211> 24
<212> DNA
<213> human Sars Coronavirus

<220>
<223> human Sars Coronavirus
primer P7

<400> 158
atgaattacc aagtcaatgg ttac 24

<210> 159
<211> 22
<212> DNA
<213> human Sars Coronavirus

<220>
<223> human Sars Coronavirus
primer P8

<400> 159
cataaccagt cggtacagct ac 22

<210> 160
<211> 16
<212> DNA
<213> human Enterovirus/human Rhinovirus

<220>
<223> primer no 28 multiplex assay

<400> 160
ctactttggg tgtccg 16

<210> 161
<211> 19
<212> DNA
<213> human Enterovirus/human Rhinovirus

<220>
<223> primer no 29 multiplex assay

<400> 161
ggtaattcca ccaccancc 19

<210> 162
<211> 23
<212> DNA
<213> human Influenza A virus

<220>
<223> probe no 30 multiplex assay

<400> 162
tcttcaagtc tctgtgcgat ctc 23

<210> 163
<211> 23
<212> DNA
<213> human Influenza B virus

<220>
<223> probe no 31 multiplex assay

<400> 163
agctcagcgc tactatattg tct 23

<210> 164
<211> 23
<212> DNA
<213> human Respiratory Syncytial virus

<220>
<223> probe no 32 multiplex assay

<400> 164
ccttccagct tgtttacata gta 23

<210> 165
<211> 23
<212> DNA
<213> human Parainfluenza 1 virus

<220>
<223> probe no 35 multiplex assay

<400> 165
aagtaccgtt tctgtcttca cat 23

<210> 166
<211> 23
<212> DNA
<213> human Parainfluenza 3 virus

<220>
<223> probe no 36 multiplex assay

<400> 166
cctgcactgt tccatcttgt ctg 23

<210> 167
<211> 18
<212> DNA
<213> human Adenovirus

<220>
<223> probe no 37 multiplex assay

<400> 167
cgcgtactcg tacaaggc 18

<210> 168
<211> 23
<212> DNA
<213> human Rhinovirus

<220>
<223> probe no 38 multiplex assay

<400> 168
attcagcata acacacaaca gcg 23

<210> 169
<211> 23
<212> DNA
<213> human Enterovirus

<220>
<223> probe no 39 multiplex assay

<400> 169
cctgtggctt cattgtcctt caa 23

<210> 170
<211> 558
<212> DNA
<213> human Herpesvirus 2

<220>

<223> modified human Herpesvirus 2
internal control
multiplex no 1

<400> 170
ttacactgtt ggttcggtgg cgactttgcc agcctgtacc ccagcatcat ccaggcccac 60
aacctgtgct tcagtacgct ctccctgcgg cccgaggccg tcgcgcacct ggaggcggac 120
cgggactacc tggagatcga ggtggggggc cgacggctgt tcttcgtgaa ggcccacgta 180
cgcgagagcc tgctgagcat cctgctgcgc gactggctgg ccatgcgaaa gcagatccgc 240
tcgcggatcc cccagagcac ccccgaggag gccgtcctcc tcgacaagca acaggccgcc 300
atcaaggtgg tgtgcaactc ggtgtacggg ttcaccgggg tgcagcacgg tcttctgccc 360
tgcctgcacg tggccgccac cgtgacgacc atcggccgcg agatgctcct cgcgacgcgc 420
gcgtacgtgc acgcgcgctg ggcggagttc gatcagctgc tggccgactt tccggaggcg 480
gccggcatgc gcgccccggg tccgtactcc atgcgcatca tctacgggga cacggactga 540
agacgtccaa aaactggc 558

<210> 171
<211> 554
<212> DNA
<213> human Herpesvirus 2

<220>
<223> modified human Herpesvirus 2
internal control
multiplex no 2

<400> 171
ctactttggg tgtccgcgac tttgccagcc tgtaccccag catcatccag gcccacaacc 60
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actacctgga gatcgaggtg gggggccgac ggctgttctt cgtgaaggcc cacgtacgcg 180
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tgcacgtggc cgccaccgtg acgaccatcg gccgcgagat gctcctcgcg acgcgcgcgt 420
acgtgcacgc gcgctgggcg gagttcgatc agctgctggc cgactttccg gaggcggccg 480
gcatgcgcgc ccccggtccg tactccatgc gcatcatcta cggggacacg gactggctgg 540
tggtggaaat tacc 554

<210> 172
<211> 23
<212> DNA
<213> human Herpesvirus

<220>
<223> Internal Control probe no 42
multiplex assay

<400> 172
acaccgagtt gcacaccacc ttg 23